

University of Windsor

Scholarship at UWindor

Electronic Theses and Dissertations

Theses, Dissertations, and Major Papers

1986

Growth hormone its mechanism of feminization of hepatic monooxygenases in male and female rats.

Birgit M. Vockentanz
University of Windsor

Follow this and additional works at: <https://scholar.uwindsor.ca/etd>

Recommended Citation

Vockentanz, Birgit M., "Growth hormone its mechanism of feminization of hepatic monooxygenases in male and female rats." (1986). *Electronic Theses and Dissertations*. 2257.
<https://scholar.uwindsor.ca/etd/2257>

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.



National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service

Services des thèses canadiennes

Ottawa, Canada
K1A 0N4

CANADIAN THESES

THÈSES CANADIENNES

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

**THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED**

**LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE**

GROWTH HORMONE: ITS MECHANISM
OF FEMINIZATION OF HEPATIC MONOOXYGENASES
IN MALE AND FEMALE RATS

by

Birgit M. Vockentanz

A Thesis
submitted to the Faculty of Graduate Studies
through the Department of
Biological Sciences in Partial Fulfillment
of the Requirements for the Degree
of Master of Science at
The University of Windsor

Windsor, Ontario, Canada

1986

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-31970-4

© Birgit M. Vockentanz 1986
All Rights Reserved

859692

For my Family

ABSTRACT

GROWTH HORMONE: ITS MECHANISM OF FEMINIZATION OF HEPATIC MONOOXYGENASES IN MALE AND FEMALE RATS

by

Birgit M. Vockentanz

It has long been known that males exhibit a 3-5 fold increased activity of certain hepatic mono-oxygenases compared to the female. These differences are due to growth hormone (GH). The purpose of this thesis was to investigate the mechanism by which this hormone decreases MFO activity in hypophysectomized rats. GH was infused via an implanted osmotic pump (1 ul/hr); the turnover of cytochrome P450 was determined by pulse labelling it with [³H]-aminolevulinic acid. In males, GH (5 ug/hr for 5 days) and puromycin (50 mg/kg, daily, for 5 days) decreased ethylmorphine demethylase (EMDM) by 27 and 40% ($p < 0.05$), respectively and each reduced cytochrome P450 by 30% ($p < 0.05$). NADPH cytochrome c reductase was increased 25-45% ($p < 0.05$). The effects of the agents together were additive. Aniline hydroxylase (ANH) was not affected. It is concluded that GH feminizes EMDM by decreasing the amount of P450. GH increased the half-life of slow phase and fast phase P450 by 67 and 80%, respectively;

the hormone decreased the amount of these phases by 54 and 16%, respectively. Microsomes from GH-treated rats showed no increase in cytochrome P450 when incubated with hemin. It is concluded that the feminization of EMDM in the male is due to a deficiency of slow phase P450 and that this results from a reduced synthesis of the apoprotein, probably effected at translation. In females, GH (10 ug/hr for 7 days) and puromycin (37 mg/kg, daily, for 5 days) each decreased both EMDM and cytochrome P450 by 31% ($p < 0.05$). Together, the effects of the agents were not additive ($p > 0.05$) and thus the site of GH action is unclear. Neither agent affected NADPH cytochrome c reductase or ANH. It is concluded that EMDM is feminized in the female also by GH effecting a decrease in cytochrome P450. GH (10 ug/hr for 7 days) did not affect the turnover rate of either fast or slow phase cytochrome P450 but it decreased the amounts by 43 and 48%, respectively ($p < 0.05$). At 7.5 ug/hr, GH decreased the amounts of these phases by 20 and 41%, respectively; EMDM was decreased by 17% ($p < 0.05$) at this dose. Microsomes from females treated with GH (10 ug/hr for 7 days) showed no increase in P450 when incubated with hemin. It is concluded that the feminization of EMDM in the female is due to a deficiency of fast phase cytochrome P450 and that this is the result of decreased synthesis of apoenzyme.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Bruce B Virgo for all the time and effort he unselfishly gave to me in preparing this manuscript. It was so very much appreciated. Only with his helpful guidance and expertise was I able to successfully complete this thesis and learn the techniques of research.

As well, I would like to thank Dr. A.H. Warner from the Department of Biological Sciences and Dr. B. Mutus from the Department of Chemistry for taking the time to review this thesis. Their criticisms and suggestions were greatly appreciated.

Finally, I would like to thank my family and friends who were always there to offer their love and support. I will always be grateful.

TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT.....	iv
ACKNOWLEDGEMENTS.....	vi
LIST OF TABLES.....	ix
LIST OF FIGURES.....	xi
INTRODUCTION.....	1

MATERIALS AND METHODS

I. General materials and methods	
A - Chemicals and Biochemicals.....	14
B - Animals.....	14
C - Growth Hormone Infusion.....	15
D - Intravenous Injection of ³ H- γ -Aminolevulinic Acid.....	16
E - Preparation of Microsomes.....	17
F - Preparation of [³ H]-Heme Labelled Microsomes for Liquid Scintillation Counting.....	18
G - Measurement of Protein Concentrations in Microsomes, Cytosol and Plasma.....	20
H - Enzyme Assays	
(i) Measurement of ethylmorphine N-demethylase..	21
(ii) Measurement of aniline hydroxylase.....	22
(iii) Quantitation of Cytochrome P450.....	24
(iv) Measurement of NADPH cytochrome c reductase.....	25
I - Quantitation of Apocytochrome P450.....	26
II. Animal Experiments	
A - Sex Differences in the Hepatic Mixed-Function Oxidase System in Male and Female Rats	
(i) Comparison of the Hepatic Mixed- Function Oxidase System in Male and Female Rats.....	27
(ii) Comparison of Hepatic Cytochrome P450 Turnover in Male and Female Rats.....	27

B - Growth Hormone (GH) and the Hepatic Monooxygenases (MFO) in the Hypophysectomized Male Rat	
(i) Growth Hormone and Puromycin: Effects on the Hepatic MFO.....	28
(ii) Turnover of Hepatic Cytochrome P450 in GH-treated Hypophysectomized Male Rats.....	29
(iii) Hepatic Apocytochrome P450 Content in GH-treated Hypophysectomized Male Rats.....	29
C - Growth Hormone (GH) and the Hepatic Monooxygenases (MFO) in the Hypophysectomized Female Rat	
(i) Growth Hormone and Puromycin: Effects on the Hepatic MFO.....	30
(ii) Turnover of Hepatic Cytochrome P450 in GH-treated Hypophysectomized Female Rats.....	31
(iii) Hepatic Apocytochrome P450 Content in GH-treated Hypophysectomized Female Rats.....	31
(iv) Puromycin and the Selective Depletion of FP-Cytochrome P450 in Hypophysectomized Female Rats....	32
D - Intact Females	
(i) Growth Hormone: Effects on the Hepatic MFO.....	32
(ii) Methyltrienolone: Effects on the Hepatic MFO.....	33
III. Statistical Analysis of the Data.....	33
RESULTS	
A. Sex Differences in the Hepatic Mixed Function Oxidase System.....	34
B. Growth Hormone (GH) and the Hepatic Monooxygenases (MFO) in the Hypophysectomized Male Rat.....	42
C. Growth Hormone (GH) and the Hepatic Monooxygenases (MFO) in the Hypophysectomized Female Rat.....	64
D. Intact Females.....	96
SUMMARY OF RESULTS.....	103
DISCUSSION.....	108
LITERATURE CITED.....	136
VITA AUCTORIS.....	146

LIST OF TABLES

<u>TABLE</u>		<u>Page</u>
I	- The mixed function oxidase system in hepatic microsomes from intact male and female rats.....	36
II	- Relative amounts of fast-phase (FP) and slow-phase (SP) cytochrome P450 in hepatic microsomes of intact male and female rats.....	41
III	- Effects of puromycin and growth hormone on body and liver weights of hypophysectomized male rats.....	44
IV	- Effects of puromycin and growth hormone on protein content in hypophysectomized male rats.....	44
V	- Effects of puromycin and growth hormone on the turnover number of hepatic microsomal ethylmorphine demethylase from hypophysectomized male rats.....	53
VI	- Effects of hypophysectomy (H _x) and growth hormone replacement on the amount of fast-phase (FP) and slow-phase (SP) hepatic microsomal cytochrome P450 in male rats.....	60
VII	- The effects of growth hormone on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats.....	65
VIII	- The effects of puromycin (25 mg/kg, daily, for 5 days and 37 mg/kg, daily, for 5 days) on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats.....	67
IX	- Effects of puromycin and growth hormone on body and liver weights in hypophysectomized female rats.....	68
X	- Effects of puromycin and growth hormone on protein content in hypophysectomized female rats.....	68

XI	- Effects of puromycin and growth hormone on the turnover number of hepatic microsomal ethylmorphine demethylase from hypophysectomized female rats.....	78
XII	- Effects of growth hormone (10 ug/hr for 5 days) on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats.....	83
XIII	- Effects of hypophysectomy (H _x) on the amount of fast-phase (FP) and slow-phase (SP) hepatic microsomal cytochrome P450 from female rats.....	87
XIV	- Effects of growth hormone (10 ug/hr for 5 days) on the amount of fast-phase (FP) and slow-phase (SP) hepatic microsomal cytochrome P450 from hypophysectomized female rats.....	88
XV	- Effects of growth hormone (7.5 ug/hr for 5 days) on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats.....	90
XVI	- Effects of growth hormone (7.5 ug/hr for 5 days) on the amount of fast-phase (FP) and slow-phase (SP) hepatic microsomal cytochrome P450 from hypophysectomized female rats.....	94
XVII	- Effects of puromycin (75 or 100 mg/kg, twice within 24 hours) on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats.....	99
XVIII	- Effects of growth hormone (10 ug/hr for 5 days) on the hepatic microsomal mixed function oxidase system from intact female rats.....	101
XIX	- Effects of methyltrienolone (125 ug/day, for 14 days) on the hepatic microsomal mixed function oxidase system from intact female rats.....	102

LIST OF FIGURES

<u>FIGURE</u>		<u>Page</u>
I	- The microsomal mixed-function oxidase in the rat liver.....	3
II	- Turnover of hepatic cytochrome P450 in intact male and female rats.....	40
III	- Effects of puromycin and growth hormone on hepatic microsomal cytochrome P450 from hypophysectomized male rats.....	47
IV	- Effects of puromycin and growth hormone on hepatic microsomal NADPH cytochrome c reductase from hypophysectomized male rats.....	49
V	- Effects of puromycin and growth hormone on ethylmorphine N-demethylase activity in hepatic microsomes from hypophysectomized male rats.....	52
VI	- Effects of puromycin and growth hormone on aniline hydroxylase activity in hepatic microsomes from hypophysectomized male rats.....	55
VII	- Cytochrome P450 turnover in hepatic microsomes from untreated (control) and GH-treated (5 ug/hr for 5 days, s.c.) hypophysectomized male rats.....	59
VIII	- Effects of growth hormone on apocytochrome P450 content in hepatic microsomes from hypophysectomized male rats.....	63
IX	- Effects of puromycin and growth hormone on hepatic microsomal cytochrome P450 from hypophysectomized female rats.....	71
X	- Effects of puromycin and growth hormone on hepatic microsomal NADPH cytochrome c reductase from hypophysectomized female rats.....	74
XI	- Effects of puromycin and growth hormone on ethylmorphine N-demethylase activity in hepatic microsomes from hypophysectomized female rats.....	77

XII	- Effects of puromycin and growth hormone on aniline hydroxylase activity in hepatic microsomes from hypophysectomized female rats.....	80
XIII	- Cytochrome P450 turnover in hepatic microsomes from untreated (control) and GH-treated (10 ug/hr for 5 days, s.c.) hypophysectomized female rats.....	85
XIV	- Cytochrome P450 turnover in hepatic microsomes from untreated (control) and GH-treated (7.5 ug/hr for 5 days, s.c.) hypophysectomized female rats.....	92
XV	- Effect of growth hormone on hepatic microsomal apocytochrome P450 content of hypophysectomized female rats.....	98

INTRODUCTION

The excretion of steroids, lipid-soluble vitamins, bile salts and other endogenous lipophilic compounds, as well as many xenobiotics, including therapeutic agents, environmental contaminants and dietary chemicals, depends upon their being made more polar. The hepatic monooxygenases or mixed function oxidases (MFO) play a major role in converting lipid soluble chemicals, which may accumulate, adversely, in the body to more water soluble metabolites.

The chemical reactions involved in such metabolism or biotransformation, can be classified into two types: phase I reactions involving oxidation, reduction or hydrolysis; and phase II reactions consisting of conjugations to polar compounds (Goth, 1984). Phase I reactions generally convert substrates to forms that are then able to undergo Phase II reactions. Most often the metabolites formed are inactive and thus their formation terminates the chemical's action (detoxification) however, in some instances the products can be more active than the parent compound (activation) and serious toxicity may ensue.

The liver, microsomal monooxygenase system can be solubilized into three functional components: 1) a family of hemoprotein isozymes, collectively termed cytochrome P450, that act as the substrate- and oxygen-

binding sites of the enzyme system; 2) NADPH cytochrome c (P450) reductase, a flavoprotein which transfers electrons from NADPH to cytochrome P450, and 3) a lipid fraction which has been identified as phosphatidylcholine and shown to be essential for electron transfer (Lu and Coon, 1968; Strobel et al., 1970). All three components are essential for a fully functional MFO system.

The proposed scheme for cytochrome P450 monooxygenase-catalyzed reactions is shown in Figure 1. In this electron transport chain, the substrate (S) combines with the oxidized form of cytochrome P450 (Fe^{+++}) to form a substrate-cytochrome P450 complex. One electron is then transferred to the substrate-P450 complex as NADPH is oxidized to NADP. This reduced (Fe^{++}) substrate-cytochrome P450 complex is able to combine with carbon monoxide to form a ferrous-CO complex with an absorbance maximum at 450 nm, thus the term cytochrome P450 originated. However, in the general reaction scheme, the reduced substrate-P450 complex reacts with molecular oxygen, subsequently undergoes a second stage of reduction (electron from NADPH) and in a series of steps that are not well understood but which are probably simple rearrangement reactions, one atom of oxygen is introduced into the substrate while the other, in the presence of two

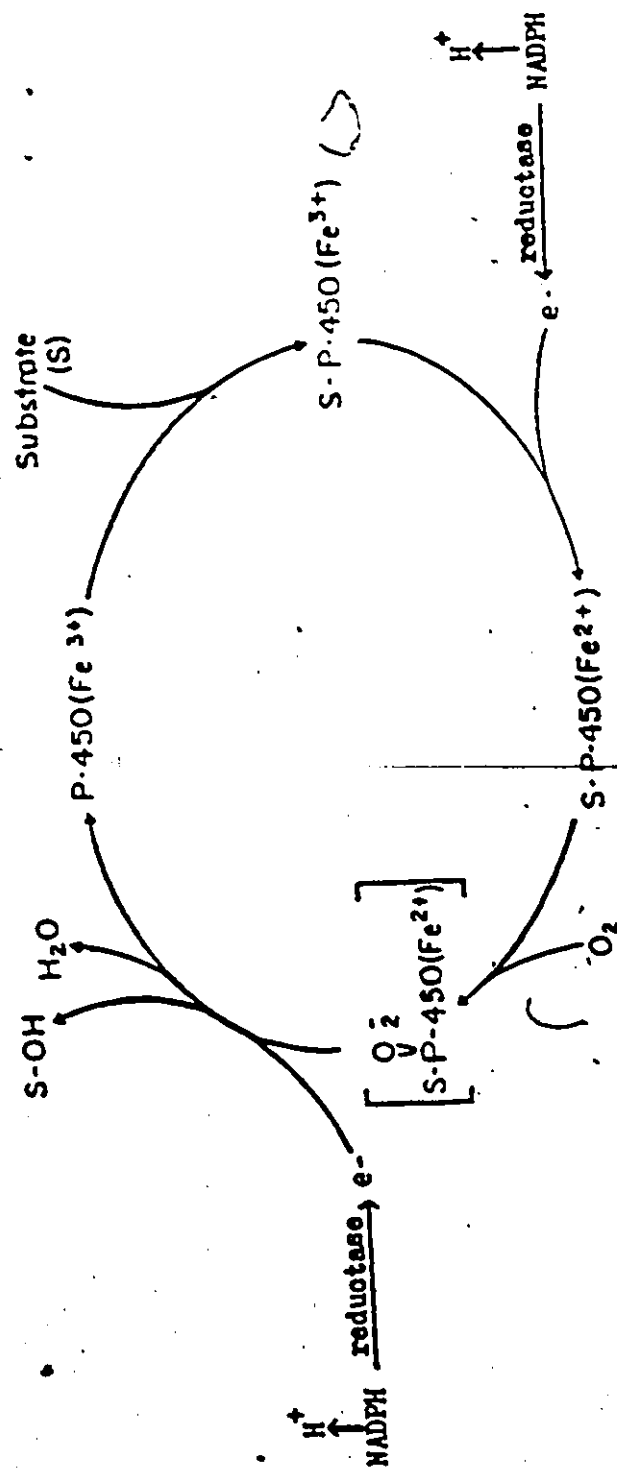


FIG. 1. General reaction scheme for cytochrome P-450-dependent mixed-function oxidase reactions. S, substrate; S-OH, oxidized substrate; e^- , electron; P-450, cytochrome P-450. Reprinted from Bend and Hook (1975).

protons, is reduced to water. The cycle is then completed with the dissociation of the hydroxylated organic substrate and the oxidation of the ferrous heme of cytochrome P450.

While the MFO system is found predominantly in the smooth endoplasmic reticulum of the liver, the kidney, lung, skin, intestine and adrenal gland also contain lower but measurable amounts of this enzyme system (Kato, 1979). It has been proposed that both cytochrome P450 and NADPH cytochrome c reductase are arranged in the ER membrane as functional clusters or patches, each comprised of 3-5 molecules of reductase and 30-500 molecules of P450 (Matsuura et al., 1978; Nebert, 1981). This arrangement is thought to facilitate efficient and direct electron transfer between the two MFO components.

The hepatic microsomal MFO catalyzes many types of oxidation reactions including aromatic and aliphatic hydroxylations; dealkylation of N-, O-, and S-alkyl compounds; desulfuration and sulfoxidation (Gillette et al., 1972; Bend and Hook, 1975). Some 16 oxidation reactions are catalyzed by P450 and all go through a hydroxylated intermediate. In addition to metabolizing drugs and other foreign compounds, the cytochrome P450 enzyme system is also involved in the metabolism of steroid hormones; that is, testosterone, estradiol and

progesterone (Welch, 1979). Since P450 exhibits a 10-100 fold higher affinity for gonadal steroids than for lipophilic xenobiotics, it has been proposed that steroid hormones serve as the physiologic substrates for liver cytochrome P450 (Waxman, 1984). Regardless of the evolution of the MFO system, what is clear is that there is a great diversity in the chemical characteristics of the various endogenous and exogenous MFO substrates. This broad substrate specificity of the hepatic MFO has been attributed, in part, to the multiplicity of P450 isozymes in liver microsomes. These P450 isozymes have different, yet overlapping substrate specificities. They differ in their immunologic and catalytic activities, electrophoretic mobility, amino acid sequences and spectral characteristics. Nine different P450 isozymes (commonly referred to as P450a, P450b, P450c, P450d, P450e, P450f, P450g, P450h, P450i) have been isolated and characterized thus far in the rat and each is the product of distinct structural genes (Ryan et al., 1984).

More than 300 compounds are known to cause proliferation of the smooth ER and to increase P450 concentrations and the activity of certain drug-metabolizing enzymes- a phenomenon referred to as induction. It is a protective adaption that eventually

leads to the enhanced clearance of the inducing agent (eg., drugs, pesticides, environmental pollutants) (Welch, 1979). The isozyme that is induced depends on the inducing agent: P450 a and e are inducible by phenobarbital; isozymes P450 c and d are induced by 3-methylcholanthrene; while P450 a-e are all inducible by Aroclor 1254, a polychlorinated biphenyl mixture (Ryan et al., 1982,1984). Therefore, the process of induction can alter the isozymic content of the MFO and concomitantly, the metabolic profile of the hepatic microsomes.

It has long been known that male and female rats differ with respect to the activities of the hepatic, microsomal drug and steroid metabolizing enzymes (Axelrod, 1956; Kato and Gillette, 1965; Gillette et al., 1972; El Defrawy El Masry and Mannering, 1974; Kato, 1974; Colby, 1980; Gustafsson et al., 1983a). In adult males, the oxidative metabolism of drugs and steroids, by P450-dependent enzymes, is generally more efficient than in adult females, whereas the opposite is true for reductive metabolism (ie., by the Δ^4 - steroid hydrogenase system). The magnitude of this sex difference, however, appears to depend on the substrate and the metabolic pathway. Thus while the rate of metabolism of ethylmorphine, aminopyrine, hexobarbital and p-nitroanisole is 3-5 fold higher in the male,

there exists no significant sex differences in the aromatic hydroxylation of zoxazolamine or aniline (Colby, 1980). The higher activity of most enzymes in the male rat is the result of the maximal enzyme velocity (V_{max}) being greater, and the apparent K_m being lesser, than in the female (Schenkman et al., 1967; Kato and Onoda, 1970). Although this sex difference in the MFO is most apparent in the rat, it has also been found to exist, to a lesser extent, in mice (where the differences are the opposite to those found in the rat) and in humans (Gustafsson et al., 1983a).

The sexual differentiation of hepatic drug and steroid metabolizing enzymes cannot be explained on the basis of varying amounts of cytochrome P450 and NADPH cytochrome c reductase. Although the levels of the two MFO components are frequently higher in livers from male rats, the differences are insufficient to account for the large sex differences in the rates of drug metabolism (Colby, 1980). Instead, what may be the underlying cause is the presence of sex-specific isozymes of cytochrome P450. While cytochromes P450a through P450e are inducible, P450f through P450i are constitutive isozymes (Ryan et al., 1982, 1984). Cytochrome P450h is male-specific and is responsible for most of the 16α -hydroxylation of progestins and androgens that is so prevalent in this sex (Waxman,

1984; Morgan et al., 1985 a,b). Cytochrome P450i is a female-specific isozyme which catalyzes the 15B - hydroxylation of steroid sulfates and is undetectable in both adult males and sexually-immature females (MacGeoch et al., 1984, 1985). Since the female P450i exhibits the lower catalytic activity towards most of the non-steroidal compounds tested with it and P450h, the existence of sex-specific P450 isozymes may provide a partial explanation for the sex differences seen. However, the isolation and purification of P450 isozymes is by no means completed and the involvement of as yet unidentified isozymes seems likely.

Sex differences in the isozymic content of hepatic, microsomal cytochrome P450 is also apparent when examining the turnover of cytochrome P450. Two distinct P450 populations can be found; one with a fast turnover rate termed the fast-phase (FP) and the other with a slow turnover rate termed the slow-phase (SP). Work by Levin and Ryan (1975) and Lui and associates (1982) has revealed that males possess more of the SP-P450 population. The isozymes that comprise these two P450 populations, however, is unknown.

The basis for the enhanced rate of enzyme activity in the male rat stems back to the involvement of testicular androgens and their dual control of hepatic drug metabolism. First, circulating androgens are

known to be important in the maintenance of a male pattern of metabolism. Castration of the adult male rat causes approximately a 30% reduction in the activity of many P450-dependent enzymes and testosterone replacement therapy will restore full activity (Kato and Onoda, 1970; El Defrawy El Masry and Mannering, 1974; Gustafsson et al., 1983a). The second aspect of hormonal control involves androgen exposure in the neonatal period. Castration of males at birth results in decreased rates of ethylmorphine, aminopyrine and hexobarbital metabolism in adulthood. Androgen treatment of these neonatally-castrated males ~~in adulthood has no effect on the MFO, however~~

testosterone administration during the neonatal period results in enzyme levels that are equal to those found in males castrated as adults (Chung et al., 1975; Chung, 1977). Indeed, treatment of the neonatally androgenized adult with testosterone restores full activity to the enzyme system. Thus, in the neonate, testicular androgens imprint a basic male pattern of metabolism and, as well, the ability to respond to androgens in adult life.

While the effects of androgens on the hepatic MFO of the male rat are well established, the role of estradiol in the female is still unclear. Although, estradiol does not appear to be necessary for a female

pattern of metabolism in the female (Colby, 1980), it does, when administered to normal or castrated males (Kramer et al., 1978a) decrease drug metabolism and feminize the MFO.

Although, androgen and estrogen receptors are found in the livers of male and both male and female rats, respectively, (Gustafsson et al., 1975; Eisenfeld et al., 1976) the steroidal control of the hepatic MFO enzymes is not effected by direct action on the liver. Gonadal hormones are unable to exert their effects on hepatic metabolism in the hypophysectomized rat and therefore, the pituitary gland must mediate their effects (Kramer et al., 1975, 1978b). Implantation of an ectopic pituitary gland under the renal capsule of hypophysectomized male rats causes feminization (decreased enzyme activities) of hepatic drug and steroid metabolism and suggests that the pituitary gland is responsible for the release of a "feminizing factor" (Gustafsson and Stenberg, 1976; Gustafsson and Skett, 1978).

Mode and associates (1983) purified this pituitary "feminizing factor" and identified it as growth hormone (GH). Extensive work with this hypophysial hormone revealed that exogenous GH feminizes both hepatic drug and steroid metabolism in intact, hypophysectomized or castrated male rat (Wilson, 1973; Kramer et al., 1978a;

Colby, 1980; Mode et al., 1981; Gustafsson et al., 1983 a,c). It is of note that while feminization of the MFO of the male is easily achieved, there is no direct evidence that GH effects feminization of drug metabolism in the female.

Since GH purified from male pituitaries has identical chemical, physical and biological properties to that purified from the female (Mode et al., 1983), it is unlikely that there exists a sex-specific form of GH which is responsible for the lower rate of metabolism in the female. However, the secretory pattern of GH is sexually-differentiated (Eden, 1979). Males exhibit large GH surges at regular 3-to 4-hour intervals with low or undetectable plasma levels between the peaks (minimum levels). In the female, on the other hand, the maximum GH levels are lower while the minimum levels are higher than in the male. Sex steroids have been shown to influence this pulsatile, sexually-differentiated secretory pattern of GH. Testosterone is necessary for the development and maintenance of high GH surges and low baseline levels in adult male rats (Jansson et al., 1984). Mode and co-workers (1982) addressed the question of how this plasma pattern of GH might regulate hepatic drug and steroid metabolism in the rat and reported that increased baseline (minimum) levels of GH appear to be

a major determinant for feminization.

The hypothalamus is probably the site at which sex steroids act to control GH release from the pituitary and hence affect enzyme activities in the MFO. Electrothermal lesions in the hypothalamus of the male rat cause feminization of hepatic metabolism. This is most likely the result of the removal of a negative hypothalamic influence and hence increased GH levels (Gustafsson et al., 1976). The hypothalamic hormone responsible for inhibiting GH release from the pituitary gland is somatostatin (SS). Work by Gross (1980) has shown that testosterone induces SS synthesis and release from the hypothalamus, thereby promoting storage of GH and inhibition of its release. It has been suggested that imprinting by neonatal testicular androgens of hypothalamic areas responsible for SS release and synthesis may increase SS levels and thus affect GH release and give males their characteristic male pattern of drug metabolism (Gustafsson et al., 1983b). The presence of steroid-binding proteins in regions of the CNS involved in GH release supports this view.

The mechanisms by which GH acts at the hepatic level to feminize microsomal P450-dependent drug metabolism is unknown. There are two GH receptors (somatogenic and lactogenic) in rat hepatocytes (Ranke

et al., 1976) and the formation of a GH-receptor complex initiates three different events; generation of a biologic signal, ligand degradation and regulated receptor loss (Hizuka et al., 1981). The purpose of this study will be to examine the effects of exogenous GH on the MFO in the hypophysectomized male and female rat and to determine the nature of the signals necessary for feminization.

MATERIALS AND METHODS

I General Materials and Methods

A. Chemicals and Biochemicals

All chemicals and enzymes were purchased from either Sigma Chemical Company (St. Louis, Missouri) or Fisher Scientific Company (Montreal, Canada) unless otherwise stated and were analytical grade (AR) or better.

Buffers were prepared, using nanopure grade distilled water, according to the methods described by Gomori (1955).

B. Animals

Rats of the Wistar strain (175-200g) (Charles River Canada, Inc., St. Constant, Quebec) were used in all experiments. Intact rats were housed in steel mesh cages suspended over absorbent paper and hardwood shavings at a constant photoperiod of 12 hours of light (lights on at 0700 hr) and a temperature of 21°C. Purina Lab Chow and tap water were provided ad libitum to all rats. Hypophysectomized rats were used 2 weeks after surgery (performed by Charles River Breeding Laboratories Inc., Wilmington, Mass.) and were supplied with a solution of 5% dextrose/0.9% NaCl as drinking water. Rats injected with ^3H - δ -aminolevulinic acid

were housed individually in disposable plastic cages (29X18X13 cm) on absorbent hardwood bedding in a separate room.

C. Growth Hormone Infusion

Growth hormone (GH) was infused continuously by means of Alzet osmotic pumps (Model 2001, Alza Corp., Palo Alto, USA), which have a nominal pumping rate of 1 μ l/hr and a nominal volume of 250 μ l. Ovine GH (NIADDK, Baltimore, MD) (Lot # AFP-4015A; AFP-4586) was dissolved in 0.03 M NaHCO₃ / 0.15 M NaCl buffer, pH 10, and the pH was adjusted to 7 with 0.2 M HCl. The solution was then loaded into the pump by use of a 1 cc syringe and a blunt needle. The flow modulator was then inserted and the pumps were stored overnight at room temperature so that they would infuse at the nominal rate immediately after implantation.

Minipump implantation was performed under light ether anaesthesia. The rat was positioned on its ventral surface, 95% ethanol was applied to the scapular region and an incision of 1-1.5 cm was made through the skin. A probe was used to separate the subdermal fascia from the skin such that a pocket was formed to receive the minipump. The wound was closed with a 9 mm stainless steel wound clip. Sham-operated rats underwent the same surgical procedure, however no

pump was inserted. All animals were then housed individually.

D. Intravenous Injection of ^3H - δ -Aminolevulinic Acid

Aminolevulinic acid (ALA) is a precursor of heme. Therefore, ^3H - δ -ALA was injected in order to label heme and consequently the hemoprotein cytochrome P450. We could then follow the turnover of the cytochrome P450 by counting the radioactivity remaining after certain time intervals. The ^3H - δ -ALA was diluted with a 0.9% NaCl solution (20 uCi/ml) and 70 uCi/kg was injected into the rats as described in the following procedure.

Animals were put under light ether anaesthesia and placed ventral side up. Both inner thighs were shaved using animal clippers and 95% ethanol was applied to the shaven area. The femoral vein was now visible and a 1-1.5 cm incision was made alongside it. Pressure was applied to the upper thigh to dilate the vein and the needle of the cannula (30 cm of intramedic polyethylene tubing, 0.38 mm ID and 1.09 mm OD) was carefully inserted into it. The ^3H - δ -ALA was then injected by means of a 1 cc syringe. The ^3H -ALA remaining in the cannula was flushed through with 250 uL of 0.9% saline. The needle was then withdrawn from the vein and firm pressure was applied to clot the blood. One or two wound clips (9 mm stainless steel)

were applied to the inner thigh to close the wound.

E. Preparation of Microsomes

Rats were weighed, decapitated and exsanguinated. The liver was excised and placed in ice-cold 50 mM Tris-HCl buffer containing 150 mM KCl, pH 7.4. All the remaining procedures were performed at 4°C. The liver tissue was cleansed (hair and blood clots) in the buffer, blotted dry and weighed. It was then minced in a diffusion glass on ice and transferred to a beaker containing three volumes of Tris-KCl. Homogenization was carried out using a Polytron PT 20 (Brinkmann Instruments Ltd., Toronto, Ontario) by placing the probe at a 45° angle and homogenizing the minced liver with 2-15 second pulses (setting at 4) separated by 30 seconds. The homogenate was then centrifuged at 9000Xg (Beckman Model J-21C centrifuge) for 20 minutes. By the use of a 10-ml pipet and a Pro-pipet, 10 ml of supernatant was carefully withdrawn from below the fat layer and transferred into 30 ml ultracentrifuge tubes. The sample was then centrifuged at 105,000Xg (Beckman Model L5-65 Ultracentrifuge) for 60 minutes. The supernatant was discarded and the pellet rinsed with 5 ml of 50 mM Tris-KCl buffer, pH 7.4. The pellet was then resuspended, with the polytron, in a volume of 50 mM Tris-KCl equal to the volume of 9000Xg supernatant that was initially centrifuged (in most cases 10 ml). The

suspension was centrifuged again at 105,000Xg for 60 minutes and the supernatant was discarded. The microsomal pellet was rinsed with 5 ml of 50 mM Tris-HCl buffer, pH 7.4 and suspended by use of the polytron in a volume of 50 mM Tris-HCl equal to one-half the volume of the 9000Xg supernatant (5 ml). This stock suspension of microsomes was diluted for all procedures which follow. The protein concentration was measured using the Lowry procedure (Lowry et al., 1951).

F. Preparation of [^3H]-Heme Labelled Microsomes for Liquid Scintillation Counting

The initial steps in microsome preparation were as described above except that the minced liver was homogenized in only 2 volumes of 50 mM Tris HCl/150 mM KCl buffer, pH 7.4. After the first ultracentrifugation (105,000Xg for 60 min. at 4°C) the resultant microsomal pellet was rinsed with 5 ml and then resuspended in 10 ml of 0.1 M potassium phosphate buffer, KH_2PO_4 - K_2HPO_4 , pH 7.4. In order to selectively solubilize and remove cytochrome b_5 , the suspension was incubated with 0.2% steapsin, under N_2 , for 1 hour, at 37°C, in a gently agitating Dubnoff incubator (GCA Corporation, Chicago, Ill.). The suspension was then centrifuged at 105,000Xg, for 60 min., at 4°C. The supernatant was discarded and the pellet washed and resuspended in 10 ml of 0.1 M K_2HPO_4 buffer, pH 7.4, followed by a final

centrifugation at 105,000Xg for 1 hour. The supernatant was again discarded and the final microsomal pellet was rinsed in situ and layered with 5 ml of 0.1 M K_2HPO_4 buffer, pH 7.4 and stored at $-20^{\circ}C$ for 1-3 days. To test the efficacy of the washing procedure, we monitored the amount of radioactivity in both the pellet and supernatant after each centrifugation. It was found that the radioactivity in the last supernatant did not differ from background levels, showing that all the unbound label was removed with the two washings. For the purpose of counting, the pellet was thawed and resuspended using the polytron. To a 20 ml plastic scintillation vial were added 0.5 ml of the suspension and 15 ml of PSC scintillation fluid (Amersham Corp., Arlington Hts., Ill.). The radioactivity was quantified using a liquid scintillation spectrophotometer (LS-3150P, Beckman Instruments Inc.). Each sample was counted for 10 min on 3 different occasions and the counts per minute (cpm) were converted to disintegrations per minute (dpm) using the external standard channels ratio method and a calibration curve, generated with 3H -ALA, microsomal protein (MP) and K_2HPO_4 buffer to correct for quenching. The microsomal protein content was determined using the Lowry procedure (see below). The data were expressed as dpm/mg microsomal protein.

G. Measurement of Protein Concentrations in Microsomes, Cytosol and Plasma.

For quantitation of microsomal protein (MP), the stock suspension of microsomes was diluted 1:100 in 50 mM Tris-HCl, pH 7.4. Protein concentrations were determined by the method of Lowry and coworkers (1951) as modified by Robson et al., (1968). Absorbance was measured against distilled water at 750 nm in a double-beam spectrophotometer (Perkin-Elmer Model 575).

Protein concentrations were estimated from a standard curve generated using bovine serum albumin as the standard protein. Standards were dissolved in 50 mM Tris-HCl, pH 7.4. Quantitation of protein in microsomes used for liquid scintillation counting was by the same procedure as above except the microsomes were suspended in 0.1 M K_2HPO_4 , pH 7.4 and another standard curve was prepared using albumin dissolved in K_2HPO_4 buffer.

Cytosolic protein concentrations were determined by diluting the supernatant of the first 105,000Xg ultracentrifugation 1:400 in 50 mM Tris-HCl, pH 7.4. Plasma protein concentrations were determined in the plasma generated after a 10 minute centrifugation (IEC HN-SII centrifuge) of the heparinized trunk blood obtained after decapitation. Plasma was diluted 1:500 in 50 mM Tris-HCl, pH 7.4.

H. Enzyme Assays

The two drug substrates whose metabolism was assessed in all experiments were ethylmorphine hydrochloride (BDH Chemical; Toronto, Ontario) and aniline (Fisher Scientific Co., Montreal, Canada) and therefore the two enzymes measured were ethylmorphine N-demethylase (EMDM) and aniline hydroxylase (ANH). The two electron transport components of the MFO were also quantified, ie., cytochrome P450 and NADPH cytochrome c reductase. Apocytochrome P450 was measured indirectly.

(i) Measurement of ethylmorphine N-demethylase

The N-demethylation of ethylmorphine was quantified by measuring the amount of formaldehyde produced by the reaction. This was done by use of the Nash reaction as described by Cochin and Axelrod (1958). First, 3 ml of incubate containing the following is prepared for each sample: 50 mM Tris-HCl (pH 7.4); 1.0 mM NADP; 3.3 mM glucose-6-phosphate; 2 units of glucose-6-phosphate dehydrogenase; 8.3 mM $MgCl_2$; 1.0 mM semicarbazide; 6.0 mg microsomal protein and 6.7 mM ethylmorphine hydrochloride (Em-HCl). A blank, containing all the components above (except EM-HCl) was also prepared for each microsomal preparation. The incubate (minus the microsomal protein) was agitated

for 5 minutes in a Dubnoff incubator (GCA Corp., Chicago, Ill.), under air, in a 25-ml Erlenmeyer flask at 37°C. Microsomal protein was then added to the flask to start the reaction which was allowed to proceed for 30 minutes. During this time, the reaction rates were linear with respect to time and protein concentration. The incubate was then added to a 15-ml Corex tube containing 3.0 ml of distilled water and the reaction terminated by the addition of 2.0 ml of 20% ZnSO_4 and 2.0 ml of saturated $\text{Ba}(\text{OH})_2$. The sample was centrifuged (IEC Model K) for 10 minutes at a setting 35 in order to precipitate the protein and $\text{Ba}_2(\text{SO}_4)_3$. A 5-ml aliquot of the supernatant was pipetted into another test-tube and 2.0 ml of double strength Nash reagent was added. All samples and blanks were then heated in a water bath at 60°C for 30 minutes. Their absorbances were then measured immediately at 415 nm, against a distilled water blank with a Perkin-Elmer 575 double-beam spectrophotometer.

(ii) Measurement of aniline hydroxylase

The hydroxylation of aniline was quantified by measuring the formation of p-aminophenol. This was done initially by the method of Kato and Gillette (1965) and then subsequently by that of Imai and coworkers (1966). For both methods, the incubate con-

tained the following: 50 mM Tris-HCl (pH 7.4); 1.0 mM NADP; 3.3 mM glucose-6-phosphate; 2 units of glucose-6-phosphate dehydrogenase; 8.3 mM $MgCl_2$; 6.0 mg microsomal protein and 4 mM aniline in a final volume of 3 ml. The incubate (minus the microsomal protein), contained in a 25-ml Erlenmeyer flask, was agitated for 5 minutes in a Dubnoff incubator (GCA Corp., Chicago, Ill.) under air, at 37°C. The reaction was allowed to proceed for 30 minutes after the addition of the microsomal protein to the flask. In the method of Kato and Gillette (1965), the incubate was added to a 60 ml bottle containing 1 g NaCl and 2.0 ml of distilled water. The blank consisted only of 1 g NaCl and 5.0 ml of distilled water. Peroxide-free anaesthetic grade, diethyl ether (30 ml) (J.T. Baker Chemical Co., Phillipsburg, N.J.) was added to each bottle, which was then capped and shaken for 10 minutes on a Elberbach reciprocating shaker (Eberbach Corp., Ann Arbor, Michigan) at 280 excursions/minute. The sample was centrifuged at setting 17 for 10 minutes in an International Centrifuge Model C50 (International Equipment Co., Boston, Mass.). Twenty-five ml of the ether were then transferred to a bottle containing 3.0 ml of 0.1 M NaOH containing 1% (w/v) phenol. The bottle was capped and shaken for 10 minutes. The mixture was then decanted into a large 25 ml test tube

and the ether aspirated off. Thirty minutes later, the absorbance of the aqueous phase was measured at 620 nm, against a distilled water blank, in a Perkin-Elmer 575 double beam spectrophotometer.

In the method of Imai et al. (1966), the 3.0 ml incubate was added to a 15-ml Corex tube containing 1.5 ml 20% tricarboxylic acid (TCA). The blank, which consisted of 3.0 ml of distilled water was treated similarly. The samples were centrifuged (IEC Model K) for 10 minutes at setting 40 and 3.0 ml of the resulting supernatants were transferred into other test-tubes. To each was added, 1.5 ml 10% Na CO and 3.0 ml of 0.2 N NaOH containing 2% phenol. The samples and blank were read immediately at 630 nm against distilled water, in a Perkin-Elmer 575 double beam spectrophotometer.

(iii) Quantitation of Cytochrome P450

Cytochrome P450 was measured by its reduced carbon monoxide difference spectrum, using $91 \text{ mM}^{-1} \text{ cm}^{-1}$ as the extinction coefficient, for the absorbance difference between 450 and 490 nm (Omura and Sato, 1964). The microsomes were diluted with 50 mM Tris-HCl, pH 7.4 to yield 6 ml of a 2 mg/ml microsomal protein solution. The sample was left at room temperature for 1-1.5 hours before being divided up into 2 cuvettes and reduced

with a small amount of sodium hydrosulfite. Both cuvettes were placed in a Perkin-Elmer 575 double beam spectrophotometer and the instrument zeroed at 400 nm. The suspensions were then scanned at a rate of 60 nm/min. (recorder speed at 10 nm/cm) from 400 to 490 nm to obtain a baseline. Carbon monoxide was then gently bubbled through the sample suspension for approximately 30 seconds and the suspensions were scanned as before from 400 to 490 nm to obtain the cytochrome P450 peak.

The total change in absorbance was the sum of the change in absorbance between the baseline and the reduced CO spectrum at 450 and 490 nm. This absorbance value was then divided by the extinction coefficient of $91 \text{ mM}^{-1} \text{ cm}^{-1}$ and the concentration of microsomal protein to yield nmoles of cytochrome P450/ mg MP.

(iv) Measurement of NADPH cytochrome c reductase.

NADPH cytochrome c reductase was assayed by the procedure of Langdon and Phillips (1962). The microsomes were diluted to a concentration of 100 ug/ml with 0.033 M phosphate buffer, pH 7.6. The reaction mixture in both the reference and sample cuvette contained 50 uM cytochrome c, 0.33 uM KCN, 0.033 M phosphate buffer, pH 7.6 and 90 ug of microsomal protein in a final volume of 2.9 ml. Both cuvettes were placed in a Perkin-Elmer 575 double beam spectrophotometer which

was then zeroed at 550 nm. To the reference cell was added 0.1 ml 0.033 M phosphate buffer (pH 7.6) and 1.1 umols NADPH solution (1 mg/0.9 ml 0.033 M phosphate buffer) was added to the sample cell. The reaction was allowed to proceed until a relatively straight line was obtained.

The slope of the resultant line was then used to calculate activity levels. The slope (change in absorbance/time) was divided by the extinction coefficient of $19.6 \text{ mM}^{-1}\text{cm}^{-1}$ and the total protein in the cuvette to yield nmols product/mg MP/minute.

I. Quantitation of Apocytochrome P450

The technique of Correia and Meyer (1975) was used to measure apocytochrome P450. Hemin was dissolved in 2.0 ml of 0.1 M NaOH and the pH was adjusted to 7.4 with 2.8 ml of 0.1 M $\text{Na}^+ - \text{K}^+$ phosphate buffer, pH 5.7. The liver, homogenized in 2 volumes of Tris-KCl, pH 7.4, was divided into 2 equal portions. Each aliquot was transferred to a 25-ml Erlenmeyer flask containing phosphatidylcholine (1mM) and phosphatidylethanolamine (0.25mM). The chloroform in which these phospholipids were originally dissolved was evaporated with a gentle stream of nitrogen gas before the microsomes were added. One aliquot was then incubated with 40 uM hemin for 20 minutes at 37°C in a Dubnoff incubator. The

other (control) aliquot was similarly incubated, however, the hemin was replaced with the buffer carrier alone. At the end of the incubation, the microsomal pellets were prepared and cytochrome P450 assayed.

II Animal Experiments

A. Sex Differences in the Hepatic Mixed Function Oxidase System

(i) Comparison of the Hepatic Mixed-Function Oxidase System in Male and Female Rats.

In order to confirm the sex differences that have been reported in the drug metabolism of the rat, we compared body weight, liver weight, microsomal protein concentration, ethylmorphine demethylase and aniline hydroxylase activity, cytochrome P450 content and NADPH cytochrome c reductase activity in male and female rats.

(ii) Comparison of Hepatic Cytochrome P450 Turnover in Male and Female Rats.

In order to measure the turnover of cytochrome P450 in male and female rats and to determine if sex differences exist in the turnover, the heme portion of the cytochrome was labelled with [3,5-³H]-delta-amino-levulinic acid (ALA) (New England Nuclear, Montreal, Que.). Rats were injected with 70 uCi/kg of -ALA

(22.4 uCi/ml of 0.9% saline) i.v. into the femoral vein under light ether anaesthesia (as described above). Four males and 4 females were then killed at each of the following times after injection: 4,8,16,24,32,48,72 and 96 hours. Microsomes were prepared for liquid scintillation counting (described above) in order to measure the amount of ^3H -P450 remaining.

B. Growth Hormone (GH) and the Hepatic Monooxygenases (MFO) in the Hypophysectomized Male Rat

(i) Growth Hormone and Puromycin: Effects on the Hepatic MFO

Growth hormone (GH) is known to feminize (decrease) hepatic drug metabolism rates in male rats. The first step in attempting elucidate the mechanism of GH action in the male was to examine the possibility that GH may exert its effects through an inhibition of protein synthesis. Therefore, 4 treatment groups were set up: hypophysectomized male (H_x) + saline (control); H_x + GH (5ug/hr, for 5 days); H_x + puromycin (P) (Boehringer Mannheim, Dorval, Quebec) (50 mg/kg, daily, for 5 days; i.p.) and H_x + GH + P. Since previous work has suggested that GH may operate at the level of translation (Korner 1968, 1969), the translational protein synthesis inhibitor, puromycin, was used. The following parameters were then quantified: body weight, liver weight, microsomal, cytosolic and plasma protein

Concentrations, EMDM, ANH, cytochrome P450 and NADPH cytochrome c reductase.

(ii) Turnover of Hepatic Cytochrome P450 in GH-treated Hypophysectomized Male Rats

From the previous experiment, it was found that GH decreased EMDM by decreasing cytochrome P450 content. This reduction in cytochrome P450 could be the result of either an increase in its rate of degradation (decrease in half-life) or a decrease in its rate of synthesis. Therefore, we wanted to determine how GH affects the half-lives of the FP- (fast turnover) and SP- (slow turnover) P450 and their relative amounts. Hypophysectomized male rats received 5 ug GH/hr for 5 days prior to the ^3H - δ -ALA injection (70uCi/kg) while controls simply underwent the surgical procedure (no GH minipump) 5 days prior to injection. The cytochrome P450 turnover curve was obtained using the method described previously for the intact animals.

(iii) Hepatic Apocytochrome P450 Content in GH-treated Hypophysectomized Male Rats

The data from the previous experiments indicated that GH decreased the synthesis of cytochrome P450. The present experiment was an attempt to determine if the decrease was the result of a decrease of apocytochrome P450 or of heme or of both. With the method of

Correia and Meyer (1975), we are able to detect the presence of any apoenzyme. If the cytochrome P450 concentration increased after incubation with hemin then originally there was free apoprotein that lacked heme. Thus, heme would have been rate-limiting in the synthesis of functional P450. Therefore, the apocytochrome levels of GH-treated hypophysectomized males were compared to sham-operated controls.

C. Growth Hormone (GH) and the Hepatic Monooxygenases (MFO) in the Hypophysectomized Female Rat

(i) Growth Hormone and Puromycin: Effects on the Hepatic MFO

The same experimental design was used for the hypophysectomized female as for the male (Section B (i)). The 4 treatment groups were set up using a GH and puromycin dosage of 5 ug/hr for 5 days and 50 mg/kg, daily, for 5 days, respectively. However, preliminary experiments revealed that 5 ug/hr for 5 days would not effect feminization in the hypophysectomized female rat while 50 mg/kg of puromycin, daily for 5 days, was close to the LD₁₀₀ for these females. Puromycin, at a dose of 25 mg/kg, had no significant effects on the MFO parameter. Therefore, for the GH and puromycin experiment in the female, GH infusion was increased to 10 ug/hr for 7 days while the puromycin dose was decreased to 37 mg/kg, daily, for 5 days.

(ii) Turnover of Hepatic Cytochrome P450 in GH-treated Hypophysectomized Female Rats

A preliminary experiment was conducted to ascertain whether a GH dose of 10 ug/hr for 5 days would yield results similar to a dose of 10 ug/hr for 7 days, the exposure used in the GH and puromycin experiment above. It was found that eliminating the last two days of treatment did not lessen the feminizing effect. Therefore, the hypophysectomized females were exposed to GH for 5 days as were the hypophysectomized males, however, the dose of GH in the female (10 ug/hr) was double that used in the male.

From the results of the turnover experiment, it was hypothesized that this GH dose (10 ug/hr for 5 days) may be at the upper limit of the dose-response curve. Therefore, we decided to use a dose of 7.5 ug/hr for 5 days (intermediate between 5 ug/hr, no effect, and 10 ug/hr, maximal effects) to examine the possibility that different GH doses have differential effects in decreasing the FP- and SP-P450 populations.

(iii) Hepatic Apocytochrome P450 Content in GH-treated Hypophysectomized Female Rats

The same procedures were followed as for the male (Section B iii). GH-treated (10 ug/hr for 5 days) hypophysectomized females were compared to sham-operated

controls with respect to the amount of apoenzyme.

(iv) Puromycin and the Selective Depletion of Fast-phase (FP) Cytochrome P450 in Hypophysectomized Female Rats

In an attempt to elucidate the metabolic functions of the FP-P450, we tried, with puromycin, to markedly decrease the amount of FP-P450 while not affecting the levels of SP-P450. We could then monitor the changes in drug metabolism that accompany the FP-P450 decline. Therefore, hypophysectomized females were treated with puromycin (75 mg/kg or 150 mg/kg, i.p.) twice within 24 hours and killed 12 hours after last injection.

Microsomes were prepared and MFO parameters assayed.

D. Intact Females

(i) The Effects of Growth Hormone on the Hepatic Mixed Function Oxidase System of Intact Females

It was decided to examine the female in a little more detail as the GH effects appeared to be more complex in this sex. Therefore, we wanted determine if GH (10ug/hr for 5 days) could feminize the MFO of intact females. Drug metabolism (EMDM and ANH) and electron-transport components (cytochrome P450 and reductase) were assayed.

(ii) The Effects of Methyltrienolone on the Hepatic Mixed Function Oxidase System of Intact Females

Methyltrienolone (New England Nuclear, Montreal, Que.), a potent synthetic androgen, was suspended in propylene glycol at a concentration of 625 ug/ml. To examine how this steroid affects the female MFO, methyltrienolone treated (125 ug/day, s.c. for 14 days) females were compared to controls (vehicle only) with respect to EMDM, ANH, cytochrome P450 and reductase activity.

III. Statistical Analysis of the Data

The data from the GH and puromycin experiments were analyzed by a 2 X 2 analyses of variance (Sokal and Rohlf, 1981). For the comparison of means, in the remaining data, the one-way anova was employed, using a level of significance of 0.05.

RESULTS

A.) Sex Differences in the Hepatic Mixed Function Oxidase System

The sex differences in the MFO, as they exist under our conditions, were determined before other experiments were conducted, and are presented in Table 1. The results obtained were comparable to those reported by others (Kato and Onoda, 1970; Kato, 1974; Colby, 1980).

The body weight of the adult male was significantly greater ($p < 0.01$) than that of the similarly aged female. The liver weight (as a proportion of body weight) and the hepatic microsomal protein concentration were, however, the same in both sexes. Therefore, there is no apparent sexual differentiation in these parameters, except for the body weight difference which is known to exist in many mammals.

However, when the metabolism of the drugs was examined, it was evident that males metabolized both ethylmorphine ($p < 0.01$) and aniline ($p < 0.05$) to a significantly greater extent than did the females. The activity of ethylmorphine demethylase (EMDM) in the male was about 200% greater than that in the female while that of aniline hydroxylase (ANH) was 31%.

greater. Therefore, the sex of the rat affects the metabolism of ethylmorphine to a greater extent than the metabolism of aniline. While EMDM is clearly a sex-differentiated enzyme, ANH, according to most (Gillette et al., 1972; Kato, 1974) is not. Even in our results the apparent sex difference in ANH was minimal. However, it was real and therefore, caused us to question the accuracy of the assay used, that of Kato and Gillette (1965). Thus, the ANH of the sexes was compared again by use of the method of Imai and coworkers (1966). Sex differences, which were quantitatively the same as those obtained with the method of Kato and Gillette, were found (Table I). Therefore, the sexual differentiation of both EMDM and ANH is indeed real in our laboratory.

If we compare the cytochrome P450 content of the liver, we see that no significant sex differences exist. Therefore, the sex differences in drug metabolism are not attributable to a greater amount of total cytochrome P450 in the male. The males do, however, exhibit a 16% higher activity of NADPH cytochrome c reductase ($p < 0.01$) but it seems unlikely that this slight increase could account for the 200% increase in EMDM activity. Therefore, the relative amounts of MFO components cannot explain the sex differences in hepatic microsomal drug metabolism.

Table I The mixed function oxidase system in hepatic microsomes from intact male and female rats. Values are the mean \pm S.E. of six rats.

Parameter	Male	Female
Body Wt. (g)	173.2 \pm 9.9	139.2 \pm 4.6**
Liver Wt. (mg/g BW)	42.7 \pm 0.87	42.9 \pm 1.26
Microsomal protein (mg/g liver)	20.25 \pm 0.85	20.42 \pm 0.54
EMDM (nmol/mg MP/min)	11.17 \pm 0.77	3.68 \pm 0.23***
ANH (nmol/mg MP/min)	0.778 \pm 0.034	0.596 \pm 0.056*
Cytochrome P450 (nmol/mg MP)	0.727 \pm 0.066	0.800 \pm 0.029
Reductase (nmol/mg MP/min)	90.0 \pm 1.98	77.8 \pm 1.27***

* p < 0.05

** p < 0.01

*** p < 0.001

From the results of these initial studies, it became apparent that there are no differences between the sexes in the total amount of cytochrome P450 in the hepatic microsomes. However, the total cytochrome P450 may be divided into two populations, one with a relatively fast turnover rate, termed the fast phase or FP-P450 and the other with a slow turnover rate termed the slow phase or SP-P450. Although total P450 is unaffected there may be sex differences in the relative amounts of these 2 populations or in their turnover rates. To test this, rats of both sexes were pulse-labelled with ^3H - δ -aminolevulinic acid, a porphobilinogen precursor which is incorporated into heme and results in the tritiated labelling of a portion of the cytochrome P450 population. The disappearance of labelled P450 was then followed.

The biphasic curve that was obtained from plotting dpm/mg microsomal protein versus time after δ -ALA injection (Figure II) was then used to determine the relative amounts of FP- and SP-P450 present at the time of injection and the half-lives of these populations. The relative amounts of FP- and SP-P450 were calculated by the method of Levin and Kuntzman (1969). Thus, the two phases of the curve were extrapolated back to zero time. The extrapolation of the uncorrected FP portion of curve yielded the total dpms of the labelled hemo-

proteins. The extrapolation of the SP portion of the curve yielded the relative amount of SP-P450. The relative amount of FP-P450 was determined by subtraction. In the determination of the half-lives, the logarithmic (base 10) values of the dpm/mg MP were plotted against time after injection. For the SP-P450, the half-life could be taken directly from the SP portion of the curve. However, to calculate the true half-life of the FP-P450, we had to generate a new graph which followed only the disappearance of FP-P450. Therefore, the dpm values of the extrapolated SP portion of curve (0,4,8,16 hr) were subtracted from the dpm values of the uncorrected FP portion of the curve (0,4,8,16 hr) and plotted (Figure I, top right). The newly generated line was used to determine the half-life of the FP-P450.

As seen in Figure II, there was a biphasic decrease in P450 in both the male and the female. As well, the half-lives of the 2 phases were similar for both sexes—about 6 hours for the FP or fast turnover type and 34 hours for the SP or slow turnover type. However, the males possessed 71% FP and 29% SP-P450 whereas the females had 81% FP and 19% SP (Table II). Thus males have more of the SP-P450 ("male-type") while females have more FP-P450 ("female-type"). Therefore, although the amount of total P450 is the same in both

Figure II Turnover of Hepatic Cytochrome P450 in
intact male and female rats.
Animals were injected with 70 uCi/kg of
 ^3H - δ -ALA (sa=1.8 mCi/nmol) and killed 4-96
hours later. Microsomes were prepared,
freed of cytochrome b₅, and their radio-
activity measured. Each point is the mean
of four rats.

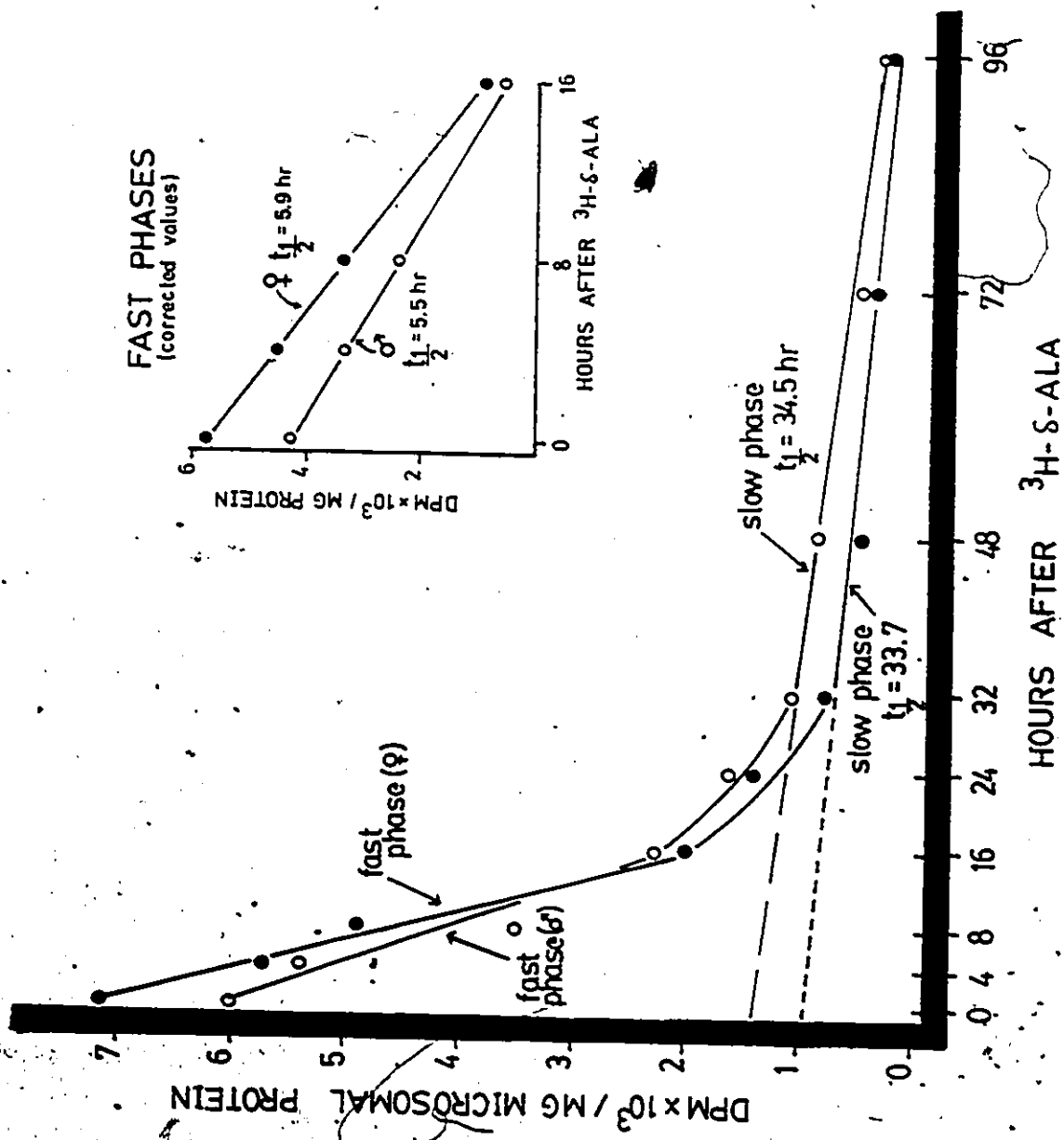


Table II Relative amounts of fast-phase (FP) and slow-phase (SP) cytochrome P450 in hepatic microsomes of intact male and female rats. Value in brackets represents the percentage of total.

	Cytochrome P450 (nmoles/mg MP)	Relative Amounts FP SP		Ratio of FP/SP fractions
Male	0.727 \pm 0.066	0.515 (70.8%)	0.212 (29.2%)	2.4:1
Female	0.800 \pm 0.029	0.646 (80.8%)	0.154 (19.2%)	4.2:1

sexes and its half-lives are the same, the FP/SP ratio increases from 2.4:1 in males to 4.2:1 in females.

B. Growth Hormone (GH) and the Hepatic Monooxygenases (MFO) in the Hypophysectomized Male Rat

(i) Growth Hormone and Puromycin: Effects on the MFO

Exogenous growth hormone feminizes hepatic drug metabolism in the intact, castrate, or hypophysectomized male rat. In an attempt to elucidate its mode of action, its effects were compared with those of puromycin, a translational inhibitor of protein synthesis. Four different treatment groups were used: one received GH (5ug/hr for 5 days), another puromycin (50mg/kg, daily, for 5 days), a third received both GH and puromycin and the fourth received only the carriers. With this experimental design, we were able to test the hypothesis that GH may act in a manner similar to puromycin, that is, by inhibiting protein synthesis at the translational level.

The body and liver weights of these rats is presented in Table III. When GH was administered, irrespective of puromycin's presence, the body weight significantly increased ($p < 0.01$). When we consider liver weight, however, GH did not cause any increase which was disproportionate to the increase in body weight. The data reveal that body weights increased

with GH but the mg liver weight/ g body weight remained relatively the same when compared to controls.

Therefore, the size of the liver must have increased proportionately with the body as would be normally expected. On the other hand, puromycin caused a 20% reduction ($p < 0.05$) in the liver weight. Therefore, at this dose, puromycin appeared to have inhibited hepatic protein synthesis. When both GH and puromycin were given, the puromycin-induced decrease in liver weight predominated over the GH-induced increase in liver weight to yield an overall decrease of 20%. But despite the reduction in liver size, all puromycin and puromycin/GH rats appeared healthy and none died over the experimental period.

From Table IV, it can be seen that neither GH nor puromycin had any significant effects on hepatic microsomal, hepatic cytosolic or plasma protein levels. However, the plasma proteins were decreased 20% by puromycin and this may be related to the significant puromycin-effected decrease in liver weight (Table III), since 85% of the plasma proteins are synthesized by hepatocytes.

The effects of GH and puromycin on cytochrome P450 and NADPH cytochrome c reductase are depicted in Figures III and IV. Total cytochrome P450 levels (Figure III) were decreased to a similar extent by both

Table III Effects of puromycin (50 mg/kg, daily, for 5 days, i.p.) and growth hormone (5 ug/hr for 5 days, s.c.) on body and liver weights of hypophysectomized male rats. Data are the means \pm S.E. of four rats.

Treatment	Body weight (g)	Liver weight (mg/g BW)
H _x + GH + P	198.75 \pm 2.32	28.33 \pm 1.90*b
H _x + -- + P	184.75 \pm 5.07	28.33 \pm 2.43*
H _x + GH + -	198.00 \pm 5.48**	35.75 \pm 2.70
H _x + saline	182.75 \pm 3.68	35.60 \pm 1.34

* p < 0.05 ** p < 0.01

b - response is due to puromycin alone

Table IV Effects of puromycin (50 mg/kg, daily, for 5 days, i.p.) and growth hormone (5 ug/hr for 5 days, s.c.) on protein content in hypophysectomized male rats. Data are the means \pm S.E. of four rats.

Treatment	Hepatic microsomal (mg/g liver)	Hepatic cytosolic (mg/ml)	Plasma (mg/ml)
H _x + GH + P	17.84 \pm 1.25	39.45 \pm 2.25	46.58 \pm 9.65
H _x + -- + P	19.08 \pm 1.77	40.20 \pm 2.99	46.85 \pm 6.51
H _x + GH + -	17.95 \pm 0.35	39.35 \pm 2.76	55.35 \pm 6.69
H _x + saline	18.61 \pm 1.20	40.95 \pm 4.00	56.90 \pm 7.82

GH (31%, $p < 0.005$) and by puromycin (33%, $p < 0.005$). The effects of GH and puromycin in combination were greater than those of GH or puromycin, individually. As well, statistical analysis revealed that there was no significant interaction between GH and puromycin, indicating that their effects were additive, and not synergistic. Additivity suggests that GH and puromycin operate through a common mechanism to decrease cytochrome P450 levels. Since puromycin inhibits protein synthesis at the translational level then GH may be operating similarly and inhibiting cytochrome P450 synthesis at the level of translation. In the case of the reductase, however, (Figure IV) GH and puromycin had effects that were the opposite to their effects on cytochrome P450. Thus, puromycin increased reductase activity by 44% ($p < 0.05$) and GH increased it by 26%, although this was not significant. Puromycin (and possibly GH) may be decreasing the synthesis of reductase degrading enzymes to a greater extent than the enzymes involved in its synthesis, thus resulting in elevated activity levels.

In Figures V and VI, the effects of GH and puromycin on hepatic drug metabolism are presented. GH caused a 27% decrease in EMDM activity ($p < 0.05$) while puromycin treatment lead to a 40% decrease in activity ($p < 0.005$). These declines in specific activity were

Figure III Effects of puromycin (50 mg/kg,daily, for 5 days,i.p.) and growth hormone (5 ug/hr for 5 days,s.c.) on hepatic microsomal cytochrome P450 from hypophysectomized male rats.

Data are the means + S.E. of four rats.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

a - response is due to the additive effects of both GH and puromycin

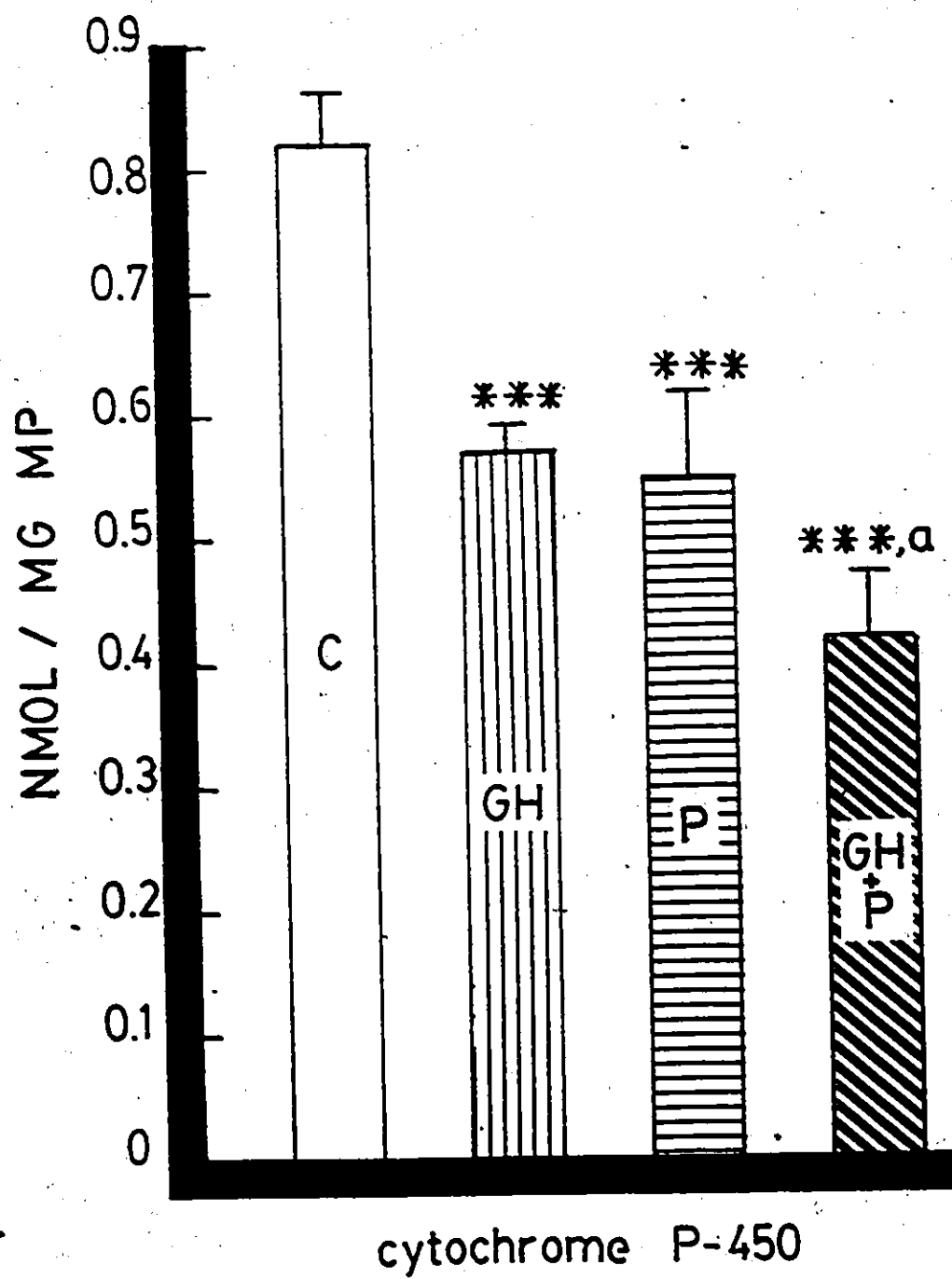
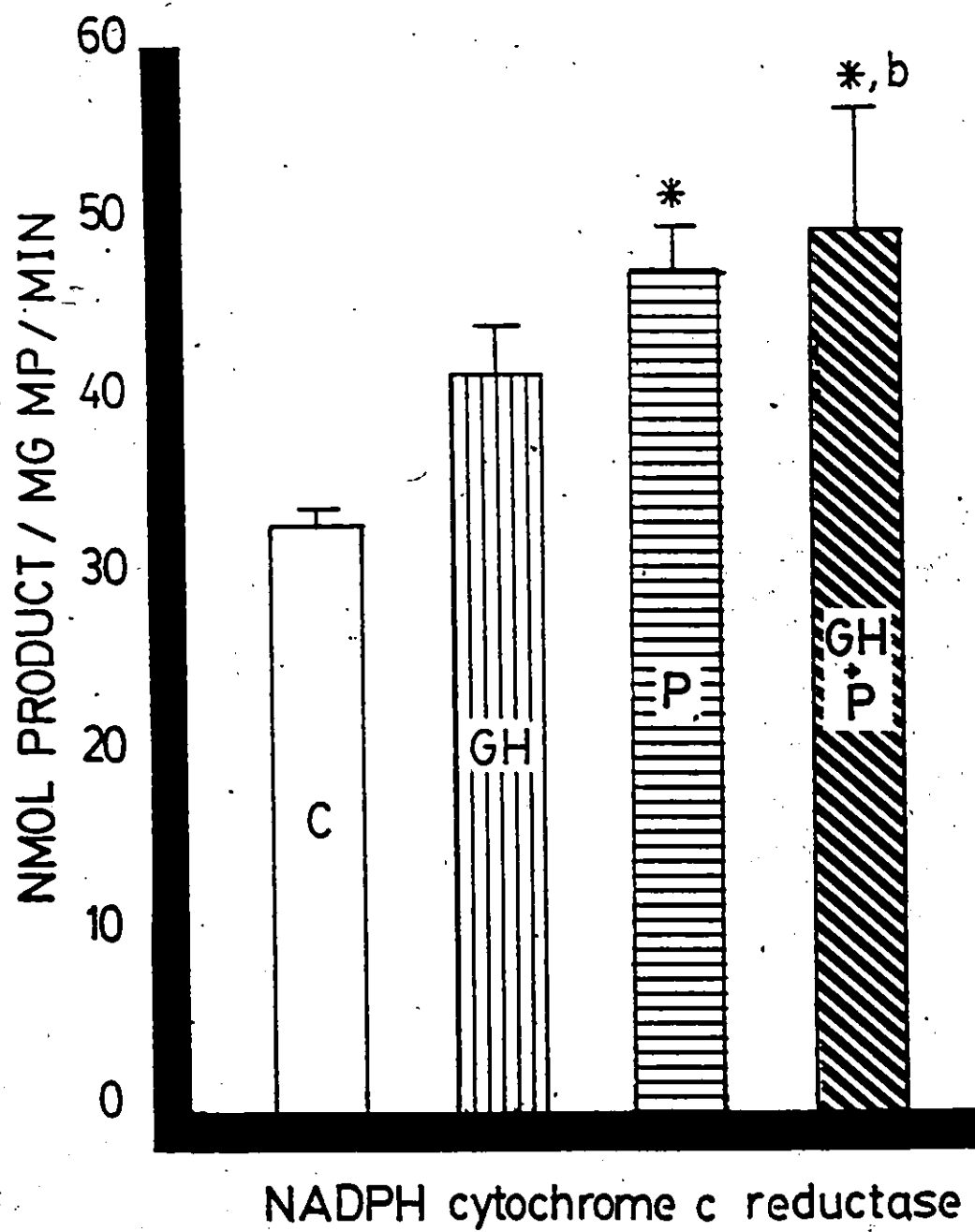


Figure IV Effects of puromycin (50 mg/kg, daily, for 5 days, i.p.) and growth hormone (5 ug/hr for 5 days, s.c.) on hepatic microsomal NADPH cytochrome c reductase from hypophysectomized male rats. Data are the means + S.E. of four rats.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

b - response is due to puromycin alone



relatable to the reductions in P450 that each agent caused (Figure III), suggesting that the decrease in ethylmorphine metabolism, caused by GH and/or puromycin, may have been the result of a decrease in cytochrome P450. Evidence to substantiate this hypothesis is found when EMDM activity is expressed in terms of cytochrome P450 (turnover number = nmol product/nmol P450/min.): the differences among the 4 treatment groups are eliminated (Table V). Also, the reduction in drug metabolism could not have been the result of a decrease in reductase activity as the specific activity was unaffected by GH and significantly increased by puromycin.

The effects of GH and puromycin on ANH activity is depicted in Figure VI. Neither GH nor puromycin had a significant effect on the specific activity of ANH. Therefore, although ANH is sex-differentiated like EMDM; it does not appear to be controlled in the same manner, ie., by the pituitary hormone GH. As mentioned previously, the fact that intact males metabolize aniline at a 30% greater rate and ethylmorphine at a 200% greater rate than females do, further supports the idea of differential control for the two drug substrates.

From this experiment, it was found that the GH-effected decrease in EMDM activity was directly

Figure V Effects of puromycin (50 mg/kg, daily, for 5 days, i.p.) and growth hormone (5 ug/hr for 5 days, s.c.) on ethylmorphine N-demethylase activity in hepatic microsomes from hypophysectomized male rats.

Data are the means + S.E. of four rats.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

a - response is due to the additive effects of both GH and puromycin

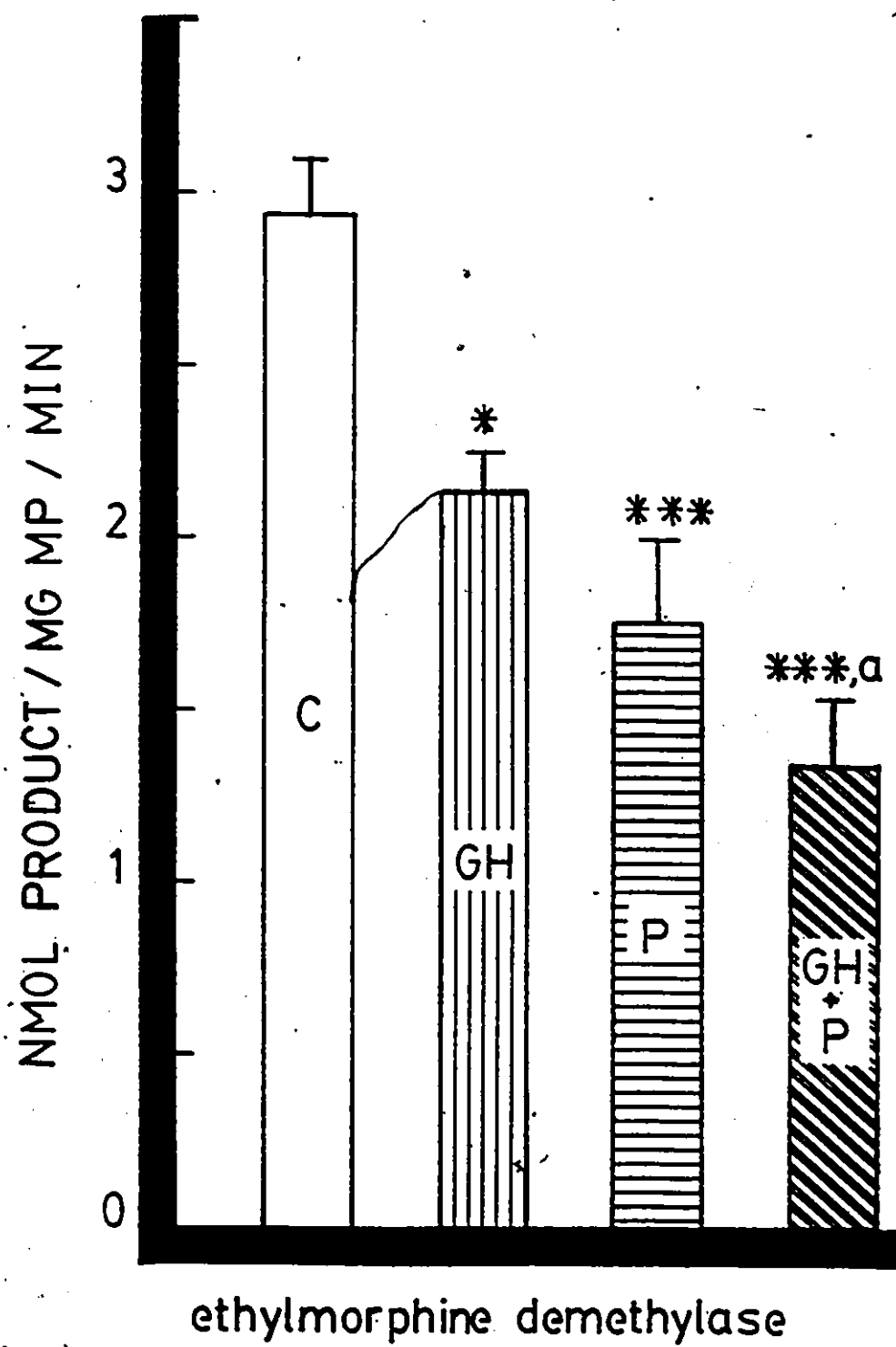
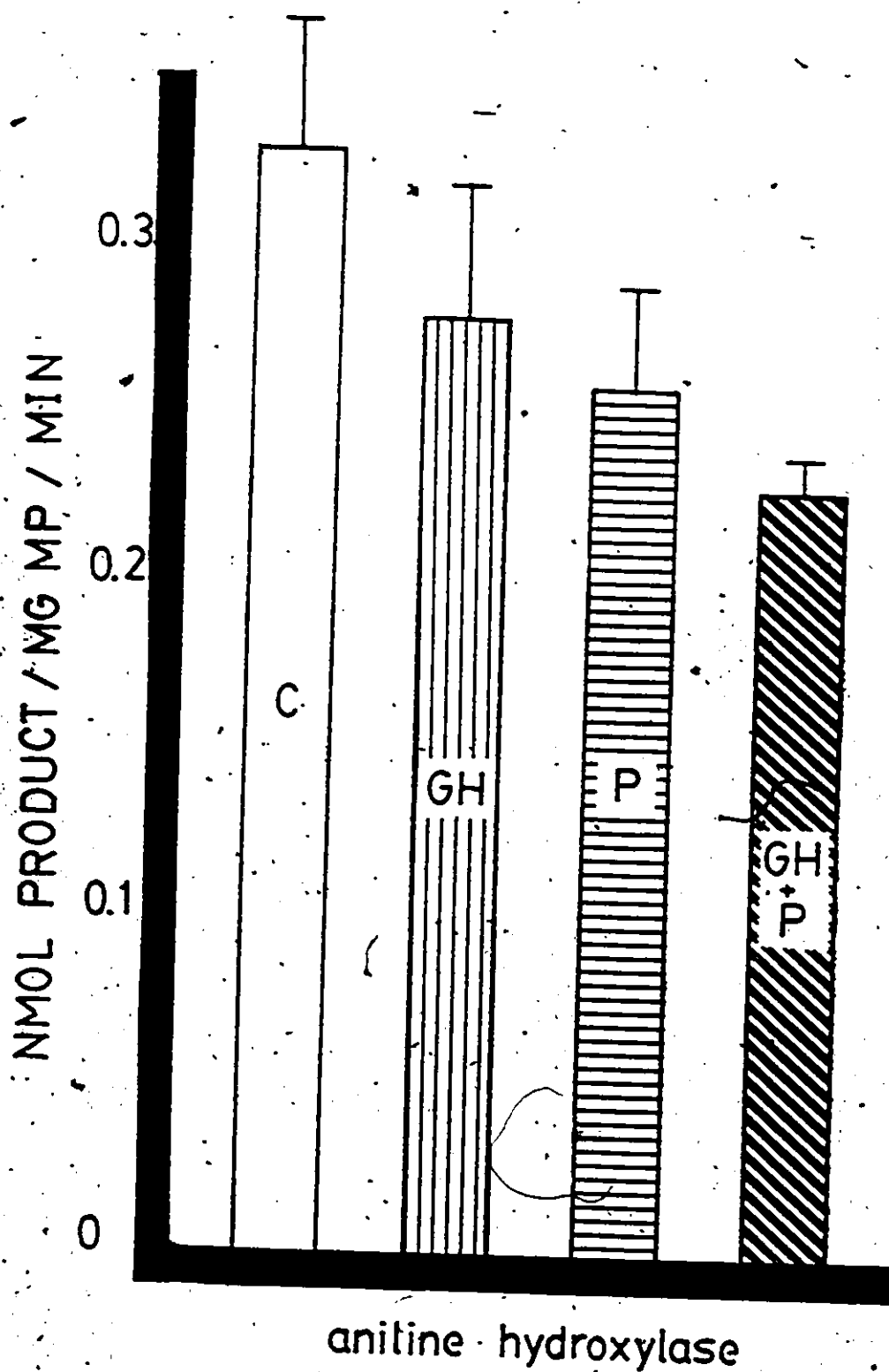


Table V Effects of puromycin (50 mg/kg,daily, for 5 days,i.p.) and growth hormone (5 ug/hr for 5 days,s.c.) on the turnover number of hepatic microsomal ethylmorphine demethylase from hypophysectomized male rats. Data are the means \pm S.E. of four rats.

Treatment	Ethylmorphine metabolism (nmol product/ nmol P450/ min.)
H _x + GH + P	3.44 \pm 0.34
H _x + -- + P	3.31 \pm 0.23
H _x + GH + -	3.75 \pm 0.02
H _x + saline	3.64 \pm 0.21

Figure VI Effects of puromycin (50 mg/kg, daily, for 5 days, i.p.) and growth hormone (5 ug/hr for 5 days, s.c.) on aniline hydroxylase activity in hepatic microsomes from hypophysectomized male rats. Data are the means + S.E. of four rats.



proportional to the decrease the hormone caused in cytochrome P450 levels. Since puromycin decreased cytochrome P450 concentrations and EMDM and its effects were statistically additive with those of GH, a common mechanism of action for GH and puromycin is suggested. Therefore, it appears that the decrease in EMDM activity was the result of the GH-effected decrease in cytochrome P450 that was due to a translational inhibition of P450 synthesis by the GH. However, we cannot, as yet, rule out the possibility that GH might also be decreasing cytochrome P450 by increasing its rate of degradation.

(ii) Turnover of Cytochrome P450 in GH-treated Hypophysectomized Male Rats.

In order to examine the possibility that GH may be increasing the rate of cytochrome P450 degradation (ie., decreasing its half-life) and consequently reducing P450 concentrations, the following experiment was carried out. Hypophysectomized males were injected with ^3H - S -ALA (70 $\mu\text{Ci/kg}$) to label the P450 and killed at various times to follow the disappearance of the labelled cytochrome P450. Males infused with GH for 5 days prior to and 1-4 days after ^3H -ALA injection, were compared to control males that received no GH. From the data it is possible to determine the half-lives of the FP- and SP-P450s as well as their relative amounts.

Figure VII shows the effects of GH on the turnover of cytochrome P450. The half-lives of the FP-P450 and SP-P450 were increased 1.8- and 1.7-fold, respectively. Both populations of cytochrome P450 were turning over at a much slower rate suggesting that GH decreased, not increased, P450 degradation. Therefore, it appears that the reduced cytochrome P450 levels that accompany the reduction in drug metabolism in the hypophysectomized male result from GH decreasing the synthesis of cytochrome P450.

The GH-effected changes in the ratio and relative amounts of the FP and SP-P450, calculated from the data in Figure VII, are presented in Table VI. Included as well in the Table are the values for the intact male (taken from Table II). The FP-P450 population was unaffected by hypophysectomy but the amount of SP-P450 was increased by almost 50%, so that the total P450 increased 13%. Hypophysectomy also resulted in a 74% reduction in EMDM activity (compare Table I and Figure V) which was clearly not the result of a decrease in cytochrome P450 but rather the result of a large decrease (63%) in NADPH cytochrome c reductase (compare Table I and Figure IV). When the hypophysectomized male was infused with GH, there was a large decrease in SP-P450 (54%) but only a slight decline in FP-P450 (16%). Therefore, from these results it appears that

Figure VII Cytochrome P450 turnover in hepatic microsomes from untreated (control) and GH-treated (5 ug/hr for 5 days, s.c.) hypophysectomized male rats. Animals were injected with 70 uCi/kg of ^3H - δ -ALA (s.a.=1.8 mCi/nmol) and killed 4-96 hours later. Microsomes were prepared, freed of cytochrome b_5 , and their radioactivity measured. Each point is the mean of four rats.

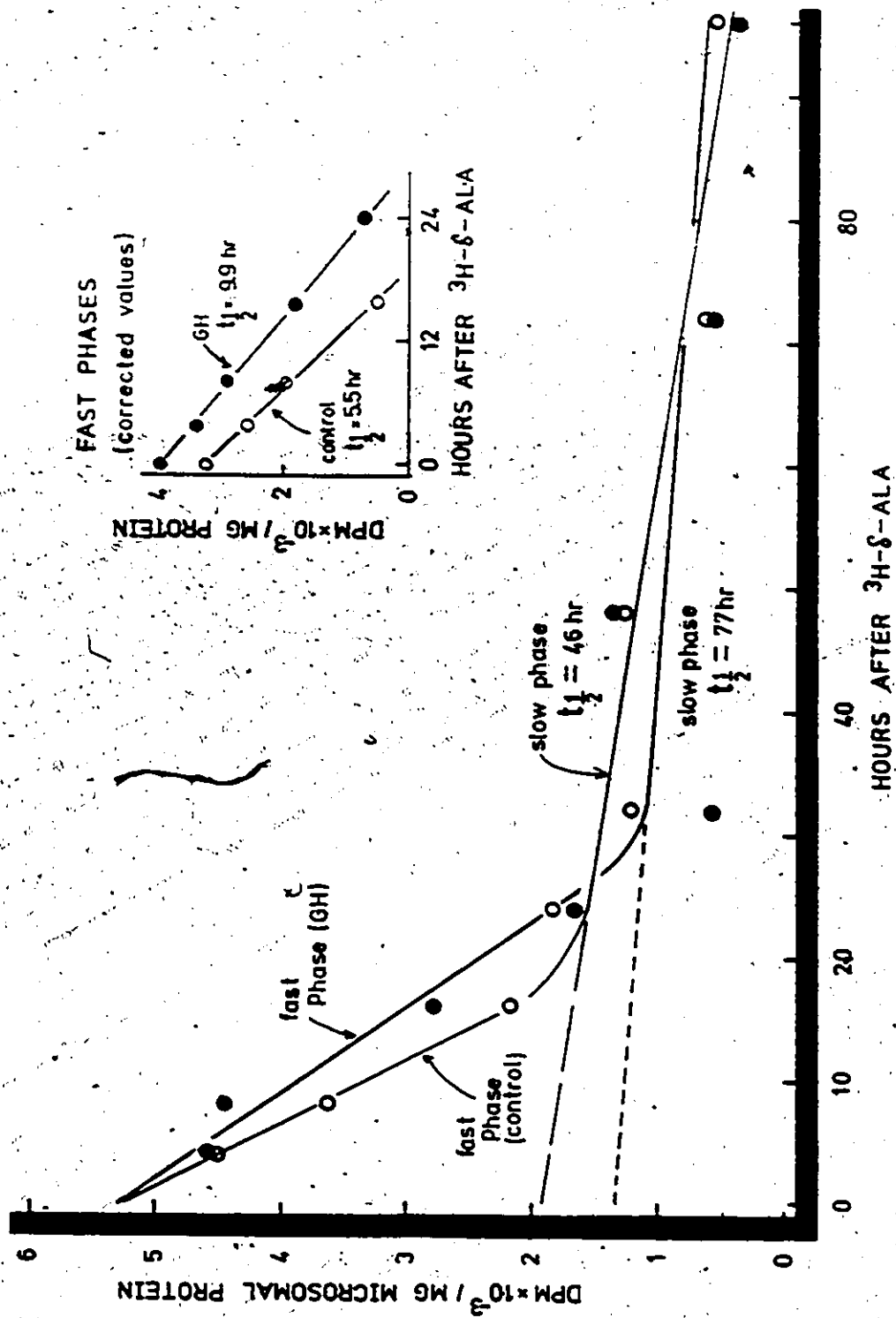


Table VI. Effects of hypophysectomy (H_x) and growth hormone replacement (5 ug/hr for 5 days) on the amount of fast-phase (FP) and slow-phase (SP) hepatic microsomal cytochrome P450 in male rats. Value in brackets represents percentage of total.

	Cytochrome P450 (nmoles/mg MP)	Relative Amounts FP SP		Ratio of FP/SP fractions
Intact*	0.727 ± 0.066	0.515 (71%)	0.212 (29%)	2.4:1
H_x	0.822 ± 0.038	0.513 (62%)	0.309 (38%)	1.7:1
$H_x + GH$	0.571 ± 0.022	0.428 (75%)	0.143 (25%)	3.0:1

* Intact male values obtained from Table II

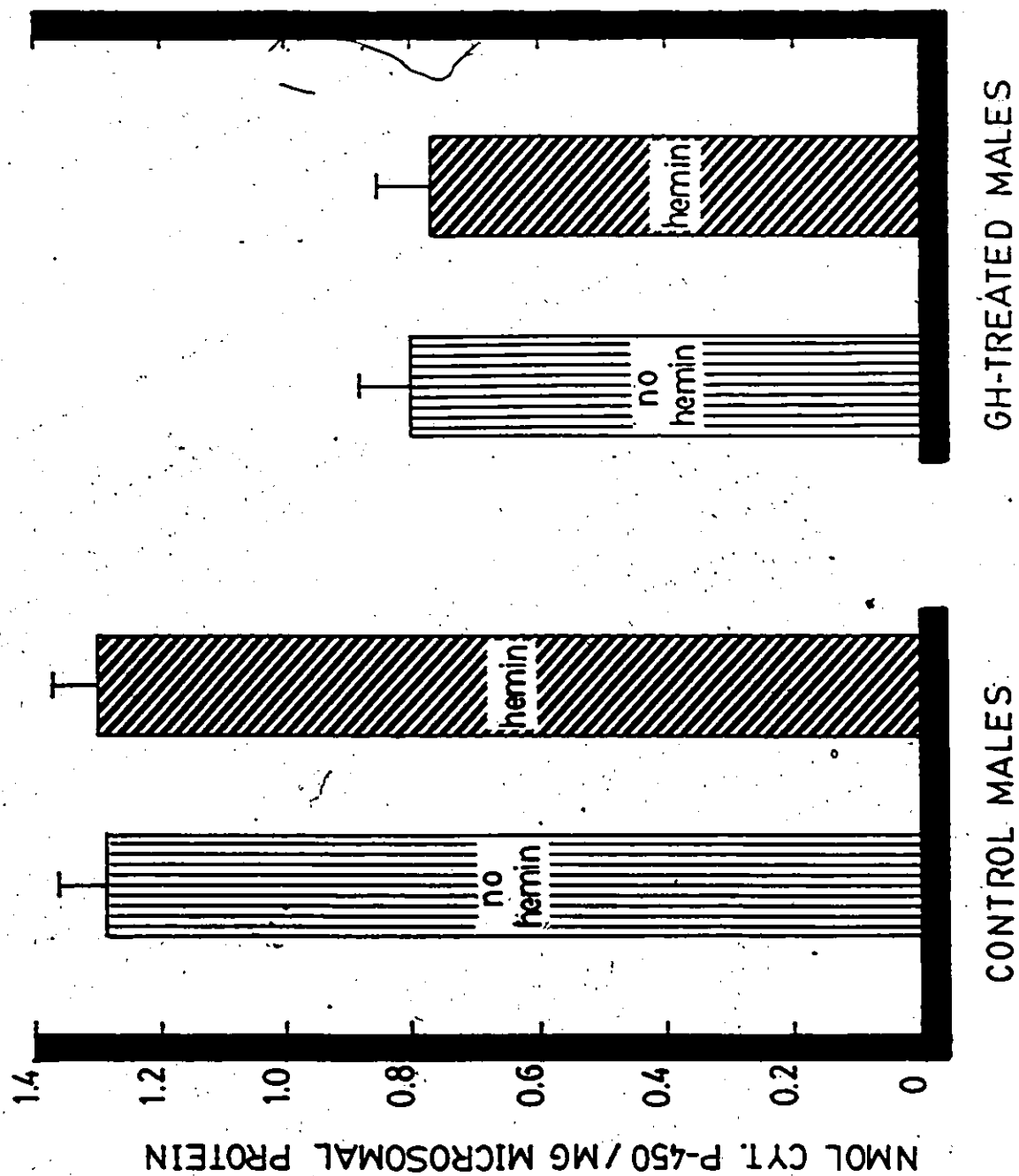
GH exerts differential control over the two P450 populations: GH suppresses the SP-P450 (population predominant in males), but exerts little or no control over the FP-P450. Consequently, GH-induced feminization of the hypophysectomized male; that is, the 27% reduction in EMDM activity, is not the result of a general decrease in cytochrome P450 but rather a selective decrease in the SP-P450 population.

(iii) Apocytochrome P450 Content in Hepatic Microsomes from GH-treated Hypophysectomized Male Rats

The decrease in cytochrome P450 synthesis which is caused by GH infusion could be due to a decrease in the synthesis of either apocytochrome P450 or heme, the two cytochrome P450 components. In order to determine which was occurring, we measured the amount of apocytochrome P450 in the hepatic microsomes of control and GH-treated hypophysectomized male rats (Figure VIII). When the microsomes of GH-infused animals were incubated with hemin, there was no significant increase in cytochrome P450 when compared to the same microsomes incubated without the hemin. Similar results were obtained for the controls. Therefore, although the total amount of cytochrome P450 was decreased by approximately 40% with GH treatment, there was no increase in the amount of apocytochrome P450. These

Figure VIII Effects of growth hormone (5 ug/hr for 5 days) on apocytochrome P450 content in hepatic microsomes from hypophysectomized male rats.

Microsomes were incubated with and without hemin and examined for an increase in cytochrome P450 levels. Data are the means + S.E. of three rats.



data would suggest that there was sufficient heme to bind all the apoenzyme and that the decrease in cytochrome P450 seen with GH treatment was the result of a decrease in the apoenzyme.

C. Growth Hormone (GH) and the Hepatic Monooxygenases (MFO) in the Hypophysectomized Female Rat

(i) Growth Hormone and Puromycin: Effects on the MFO

The results from the experiments above suggest that GH feminizes drug metabolism in the hypophysectomized male by decreasing the synthesis of SP-apocytochrome P450 at the translational level. In an attempt to determine if GH operates in a similar manner in the hypophysectomized female, the same experimental design that was used for the male was applied to the female.

However, when the same doses were used in the female, the effects were different. It is evident from Table VII that a GH infusion rate of 5 ug/hr for 5 days was inadequate and unable to produce a feminizing effect in the hypophysectomized female. None of the MFO parameters were significantly different from control values. Therefore, the dose was doubled, the exposure time lengthened and the experiment repeated. Experiments with 10 ug/hr for 7 days (Table VII) indicated that this dose caused a feminization

Table VII The effects of growth hormone on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats. Data are the means \pm S.E. of three rats.

Parameter	Growth Hormone		Controls
	(5 ug/hr/ for 5 days)	(10 ug/hr/ for 7 days)	
Body Wt. (g)	194 \pm 4.0	190 \pm 1.5	180 \pm 3.5
Liver Wt. (mg/g BW)	36.3 \pm 0.35	35.7 \pm 1.71	37.9 \pm 1.17
Microsomal protein (mg/g liver)	13.1 \pm 0.45	15.4 \pm 1.76	15.6 \pm 0.78
EMDM (nmol/mg MP/min)	3.21 \pm 0.27	1.64 \pm 0.24*	3.05 \pm 0.29
ANH (nmol/mg MP/min)	0.603 \pm 0.010	0.440 \pm 0.089	0.569 \pm 0.009
Cytochrome P450 (nmol/mg MP)	0.921 \pm 0.047	0.569 \pm 0.058**	0.941 \pm 0.044
Reductase (nmol/mg MP/min)	37.7 \pm 0.60	38.4 \pm 6.32	34.0 \pm 2.42

* p < 0.05

** p < 0.01

comparable to that found in the male, albeit at half the dose.

Similarly, the female did not respond to puromycin in the same manner as the male. Thus, 50 mg/kg, daily, for 5 days, as used in the male, caused 100% mortality in hypophysectomized female rats. Therefore, the dose was decreased to 25 mg/kg, daily, for 5 days and as seen in Table VIII, this dose caused only a slight decrease in liver weight. However, a puromycin dose of 37 mg/kg, daily, for 5 days caused a decrease in liver weight, cytosolic and plasma protein levels, and, as well, a decrease in drug metabolism (EMDM and ANH) and cytochrome P450. Therefore, this dose was used for the GH and puromycin experiment. The necessity of increasing the GH dose and decreasing the puromycin dose in the female suggests a sex difference in either the mechanisms involved in feminization or in the sensitivity of the MFO system, or possibly both.

The effects of GH and/or puromycin on body and liver weight are shown in Table IX. GH caused a significant increase (14%) in body weight ($p < 0.01$) indicating that the GH was efficacious in the hormone-deficient females. The absence of any body weight loss in the rats treated with puromycin suggests that its detrimental effects were not severe and thus were not adversely affecting the animals. This dose of

Table VIII The effects of puromycin (25 mg/kg, daily, for 5 days and 37 mg/kg, daily, for 5 days) on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats.
Data are the means \pm S.E. of three rats.

Parameter	Puromycin		Controls
	(25 mg/kg, daily, for 5 days)	(37 mg/kg, daily, for 5 days)	
Body Wt. (g)	177 \pm 7.0	166 \pm 5.0	180 \pm 3.5
Liver Wt. (mg/g BW)	30.6 \pm 2.00*	28.7 \pm 0.85**	37.9 \pm 1.17
Microsomal protein (mg/g liver)	15.1 \pm 0.70	13.1 \pm 0.15	15.6 \pm 0.78
Cytosolic protein (mg/ml)	31.0 \pm 0.60	23.0 \pm 0.45**	36.3 \pm 1.89
Plasma protein (mg/ml)	45.5 \pm 1.20	39.2 \pm 0.80*	52.4 \pm 3.27
EMDM (nmol/mg ^a MP/min)	2.61 \pm 0.13	1.83 \pm 0.09*	3.05 \pm 0.29
ANH (nmol/mg MP/min)	0.496 \pm 0.044	0.439 \pm 0.012**	0.569 \pm 0.009
Cytochrome P450 (nmol/mg MP)	0.789 \pm 0.091	0.610 \pm 0.006**	0.941 \pm 0.044
Reductase (nmol/mg MP/min)	30.2 \pm 1.70	39.3 \pm 0.65	34.0 \pm 2.42

* p < 0.05

** p < 0.01

Table IX Effects of puromycin (37 mg/kg, daily, for 5 days, i.p.) and growth hormone (10 ug/hr for 7 days, s.c.) on body and liver weights in hypophysectomized female rats. Data are the means \pm S.E. of five rats.

Treatment	Body weight (g)	Liver weight (mg/g BW)
H _x + GH + P	171.2 \pm 2.13	29.6 \pm 1.46***b
H _x + -- + P	161.2 \pm 4.60	34.8 \pm 3.54***
H _x + GH + -	173.0 \pm 7.00**	48.2 \pm 4.10
H _x + saline	151.8 \pm 4.73	48.6 \pm 3.86

p < 0.05 ** p < 0.01 * p < 0.001
b- response is due to puromycin alone

Table X Effects of puromycin (37 mg/kg, daily, for 5 days, i.p.) and growth hormone (10 ug/hr for 7 days, s.c.) on protein content in hypophysectomized female rats. Data are the means \pm S.E. of five rats.

Treatment	Hepatic microsomal (mg/ g liver)	Hepatic cytosolic (mg/ml)	Plasma (mg/ml)
H _x + GH + P	14.3 \pm 0.59	24.6 \pm 1.2	42.3 \pm 1.42
H _x + -- + P	13.4 \pm 0.93	22.7 \pm 2.06	39.6 \pm 1.28***
H _x + GH + -	14.4 \pm 0.98	26.8 \pm 2.78	50.9 \pm 3.56
H _x + saline	14.2 \pm 0.74	25.0 \pm 2.14	48.6 \pm 3.52

* p < 0.05 ** p < 0.01 *** p < 0.001

puromycin did however affect the liver, as indicated by a 28% drop in weight ($p < 0.001$). As found in the males, GH caused liver growth with the liver weight increasing from 7.38 g in controls to 8.34 g in the GH-treated rats, an increase of 13%. This GH-effected growth maintained the normal liver weight to body weight ratio. However, when puromycin was simultaneously administered with GH, the liver weight decreased (39%, $p < 0.001$).

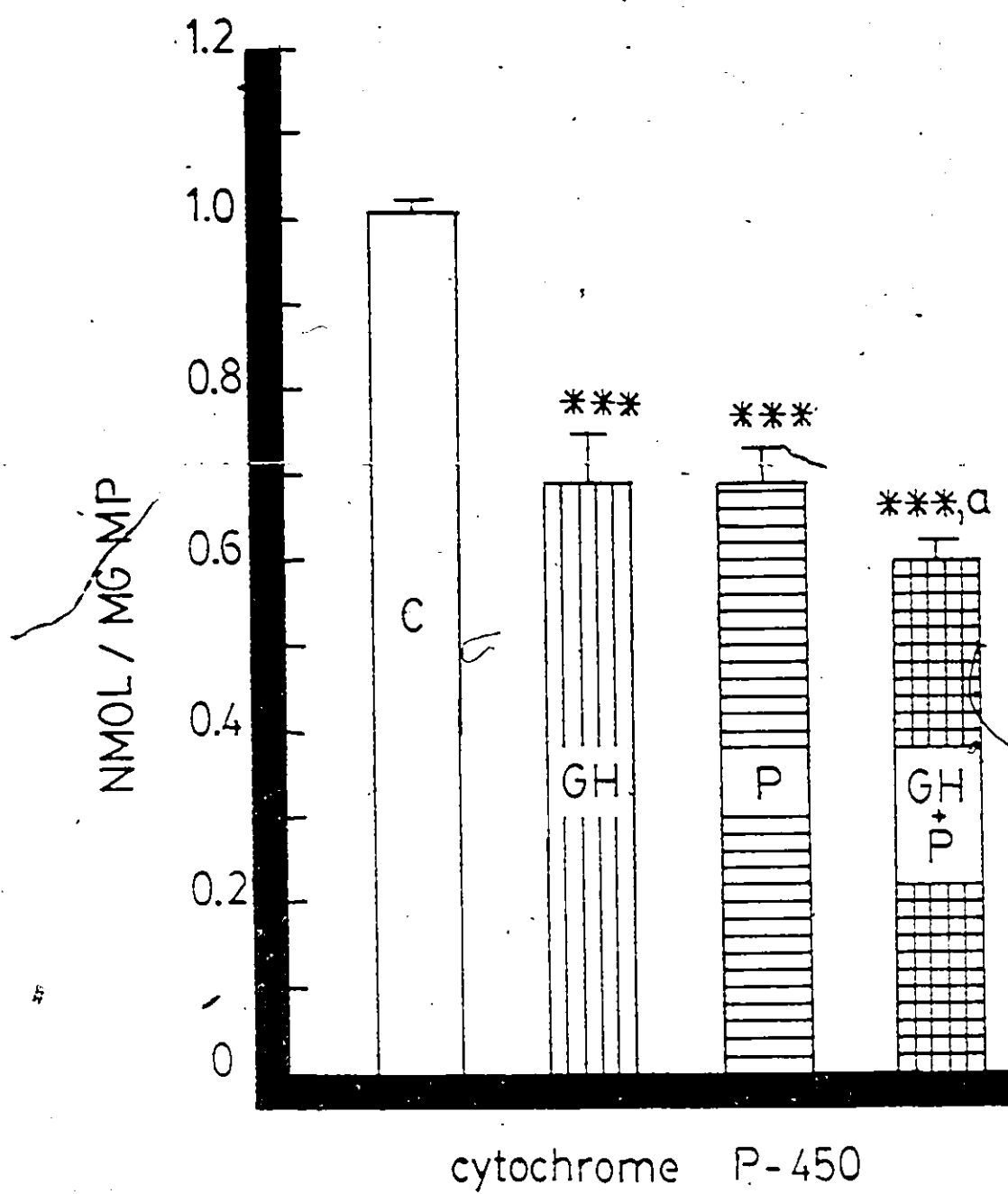
The effects of GH and puromycin on tissue protein concentrations are summarized in Table X. While neither treatment significantly affected the amounts of hepatic microsomal or cytosolic protein, puromycin significantly ($p < 0.01$)-decreased plasma protein concentrations (19%). It is not surprising that there was a similar trend for plasma proteins and liver weight (Table IX) since the liver synthesizes about 85% of these proteins.

Both puromycin and GH significantly lowered cytochrome P450 concentrations (Figure IX). At the doses used, the reduction effected by both agents was equivalent, 31% ($p < 0.001$). Thus, GH and puromycin had similar effects on P450, as was the case in the male. However, in the females there was a significant interaction effect ($p < 0.01$) when both agents were given together. This indicates that their individual effects

Figure IX Effects of puromycin (37 mg/kg, daily, for 5 days, i.p.) and growth hormone (10 ug/hr for 7 days, s.c.) on hepatic microsomal cytochrome P450 from hypophysectomized female rats. Data are the means \pm S.E. of five rats.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

a - response is due to the non-additive effects of both GH and puromycin

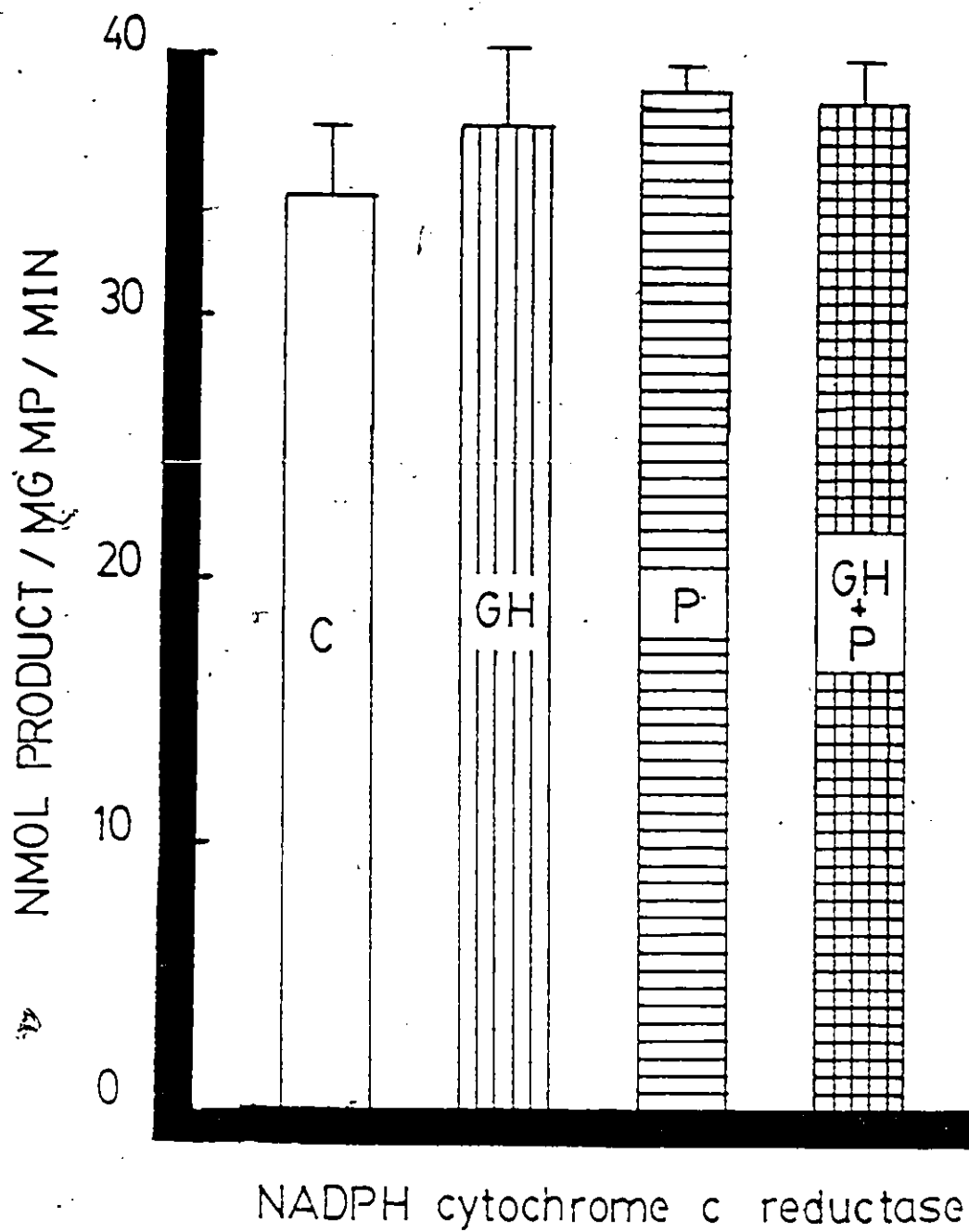


may not be additive which implies that GH and puromycin may act via different mechanisms to decrease the amount of cytochrome P450. Since puromycin operates at the level of translation to decrease protein synthesis, several possible causes exist for the interaction effect seen. The interaction may mean that GH acts only at non-translational sites or it may mean that it acts, simultaneously, at translation and at least one other site. On the other hand, the interaction may simply reflect the possibility that the GH and puromycin doses are sufficiently high to cause essentially maximal effects, so that the two together can cause no further reduction in the levels of cytochrome P450. Additivity may, therefore, be masked by a dose effect. Evidence to substantiate this possibility comes from the Simple Effects Test (Keppel, 1982): thus, there were significant differences when either GH or puromycin effects were compared with the controls (no treatment), but there were no differences when either the GH or puromycin effects were compared with the effects from the combination of GH and puromycin.

The effects of GH and puromycin on the other electron-transport component of the MFO, NADPH cytochrome c reductase, is shown in Figure X. Neither treatment had any significant effect on the specific activity of the reductase. Therefore, any changes in

Figure X Effects of puromycin (37 mg/kg, daily, for 5 days, i.p.) and growth hormone (10 ug/hr for 7 days, s.c.) on hepatic microsomal NADPH cytochrome c reductase from hypophysectomized female rats. Data are the means + S.E. of five rats.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$



drug metabolism cannot be attributed to a change in reductase activity.

The drug metabolism parameters are presented in Figures XI and XII. EMDM activity was reduced by both puromycin (28%, $p < 0.01$) and GH (31%, $p < 0.01$) (Figure XI). These decreases were comparable to the 31% reduction in cytochrome P450 levels caused when either agent was administered. Such parallel changes in EMDM activity and cytochrome P450 levels suggest that the decrease in ethylmorphine metabolism may result from the decrease in cytochrome P450. Support for this view is obtained when ethylmorphine metabolism is expressed in terms of cytochrome P450 (nmol product/ nmol cytochrome P450/ min.): the differences between the controls and GH-treated rats are eliminated (Table XI). As well, the fact that NADPH cytochrome c reductase is unaffected by GH (Figure X) and thus is not the cause of any decrease in EMDM activity further supports the idea of the decrease in drug metabolism being due to a decrease in cytochrome P450 content. As in the males, there was no interaction between GH and puromycin, thus supporting the view that there is a common mechanism of action for the two agents; that is, both GH and puromycin decrease the activity of EMDM by decreasing P450 levels.

The effects of GH and puromycin on ANH activity

Figure XI Effects of puromycin (37 mg/kg, daily, for 5 days;i.p.) and growth hormone (10 ug/hr for 7 days;s.c.) on ethylmorphine N-demethylase activity in hepatic microsomes from hypophysectomized female rats. Data are the means + S.E. of five rats.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

a- response is due to the additive effects of both GH and puromycin

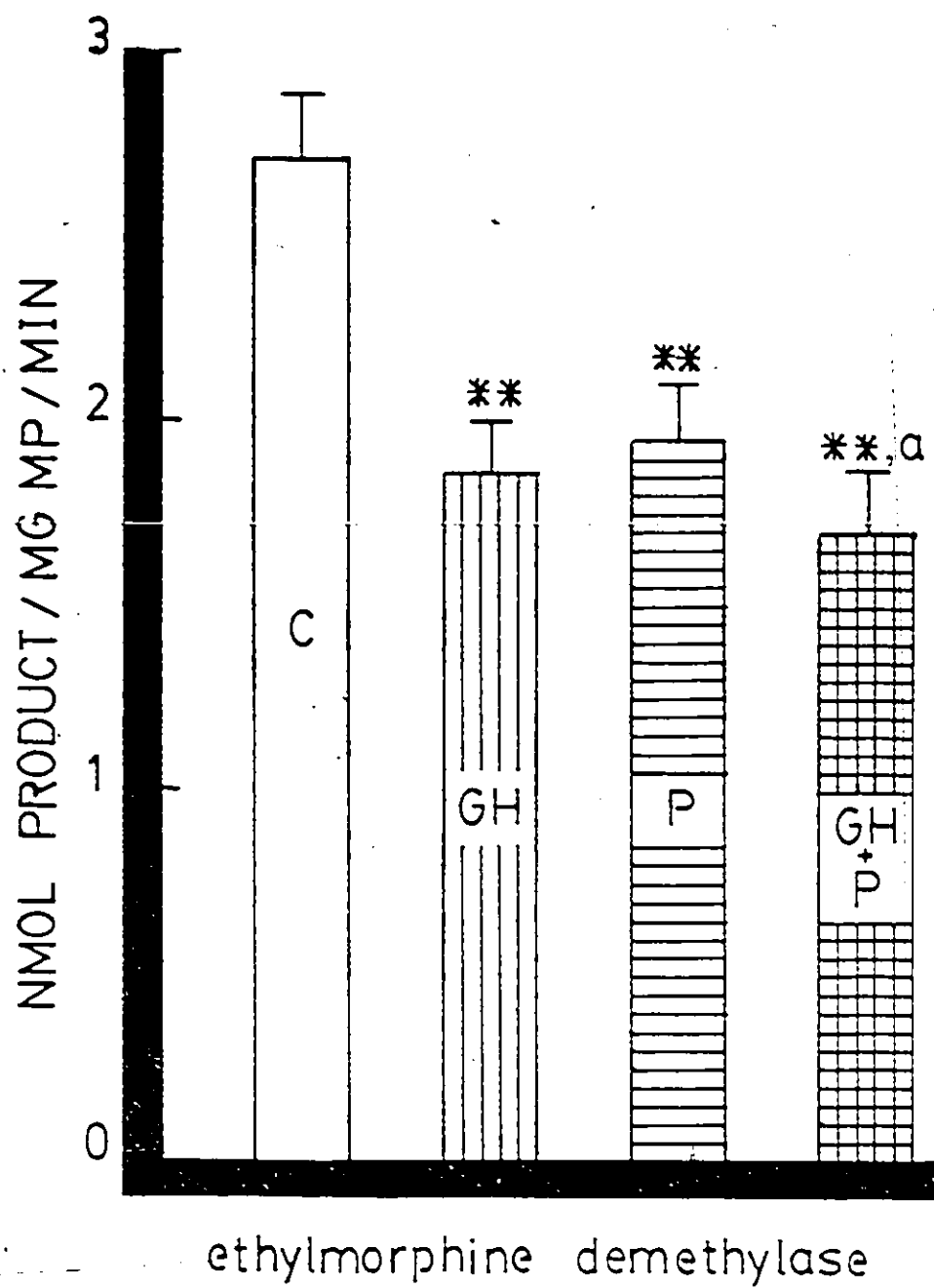
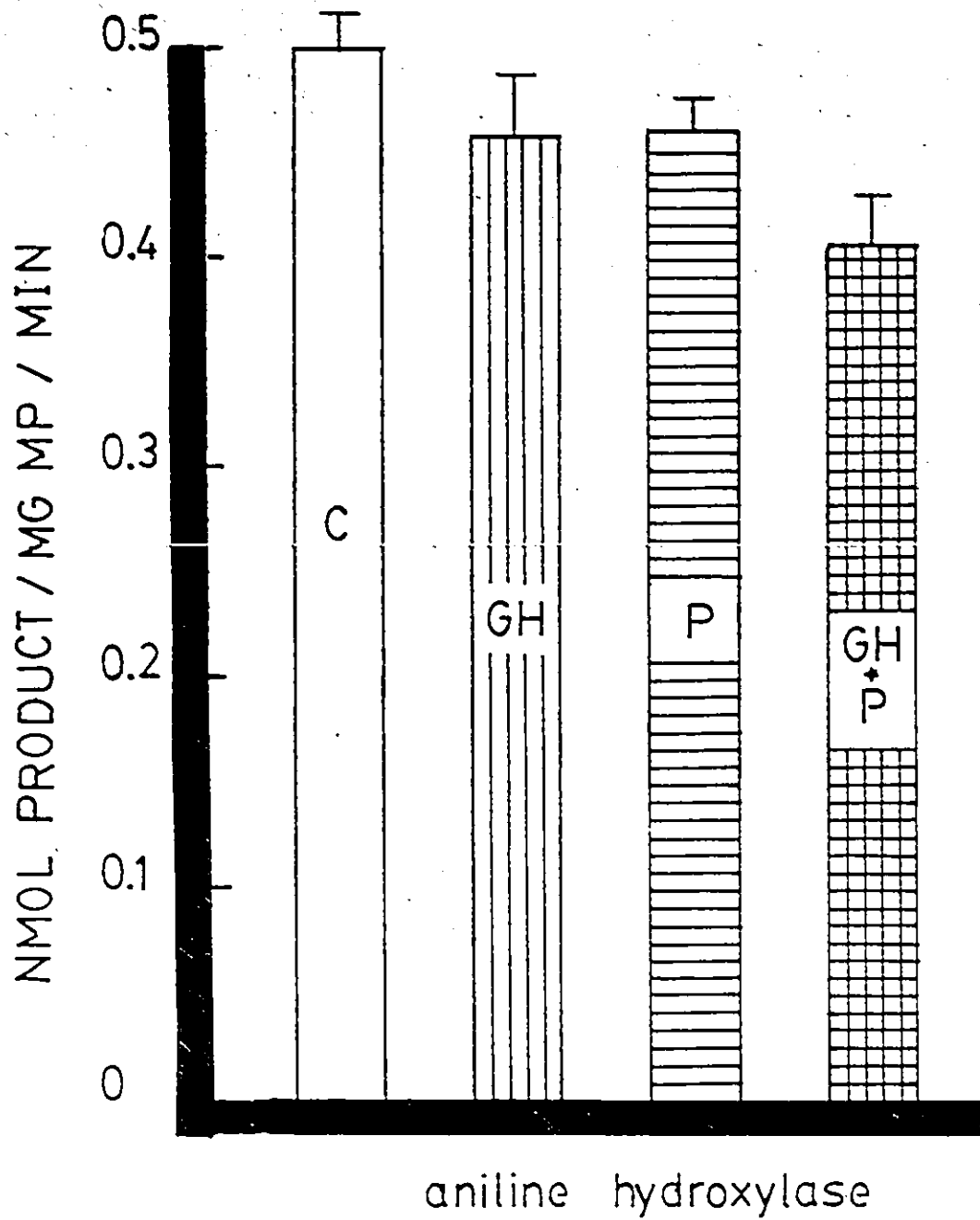


Table XI Effects of puromycin (37 mg/kg; daily, for 5 days, i.p.) and growth hormone (10 ug/hr for 7 days, s.c.) on the turnover number of hepatic microsomal ethylmorphine demethylase from hypophysectomized female rats. Data are the means \pm S.E. of five rats.

Treatment	Ethylmorphine metabolism (nmol product/ nmol P450/ min.)
H _x + GH + P	2.80 \pm 0.23
H _x + -- + P	2.83 \pm 0.19
H _x + GH + -	2.72 \pm 0.11
H _x + saline	2.70 \pm 0.17

Figure XII Effects of puromycin (37 mg/kg, daily, for 5 days;i.p.) and growth hormone (10 ug/hr for 7 days;s.c.) on aniline hydroxylase activity in hepatic microsomes from hypophysectomized female rats. Data are the mean + S.E. of five rats.



are depicted in Figure XII. It was found that neither agent had any significant effect on aniline metabolism. Therefore, GH in the females does not appear to be responsible for controlling the sex difference in ANH. The same observation was made previously for the males.

From the results of the experiments with GH and puromycin, it is apparent that GH decreases (feminizes) drug metabolism in the hypophysectomized female through a decrease in cytochrome P450. Although, both GH and puromycin decrease P450 levels, the evidence suggests that GH may not effect this solely through a translational inhibition of protein synthesis, as does puromycin. Instead, GH may be operating at additional or completely different sites to lower P450 in the hypophysectomized female. Thus, whether GH causes a decrease in the rate of cytochrome P450 synthesis or an increase in its rate of degradation was to be determined in the following experiment.

(ii) Turnover of Cytochrome P450 in GH-treated Hypophysectomized Female Rats

To study the mode of GH action and to determine whether decreased synthesis or increased degradation is responsible for the depressed cytochrome P450 levels in the hypophysectomized female, the turnover of cytochrome P450 was examined. The P450 of control and GH-treated hypophysectomized females was labelled by

the use of ^3H - γ -ALA (70 uCi/kg) and the rats were killed at various time intervals to measure the radioactivity remaining in the microsomes. Half-lives and the relative amounts of FP- and SP-P450 were calculated from the resulting data.

In the females of the GH X puromycin experiment, a GH dose of 10 ug/hr for 7 days was used. In the studies with males the duration of GH exposure was only 5 days. In order to obtain data more readily comparable between the sexes, the GH treatment period in this experiment was reduced to 5 days. Preliminary experiments with 10 ug/hr for 5 days demonstrated that feminization still occurred with GH significantly decreasing both EMDM activity (48%, $p < 0.01$) and cytochrome P450 levels (45%, $p < 0.01$) (Table XII).

The effects of GH (10 ug/hr for 5 days) on the half-lives of FP- and SP-cytochrome P450 are shown in Figure XIII. The half-lives of both FP- and SP-P450 were essentially unaffected by the GH treatment. Thus, in the hypophysectomized female, as in the male, decreased cytochrome P450 synthesis appears to be the major mechanism by which GH reduces cytochrome P450 levels.

Before examining the effects of GH treatment on the amounts of FP- and SP-P450, we should first consider the effects of hypophysectomy; that is, the

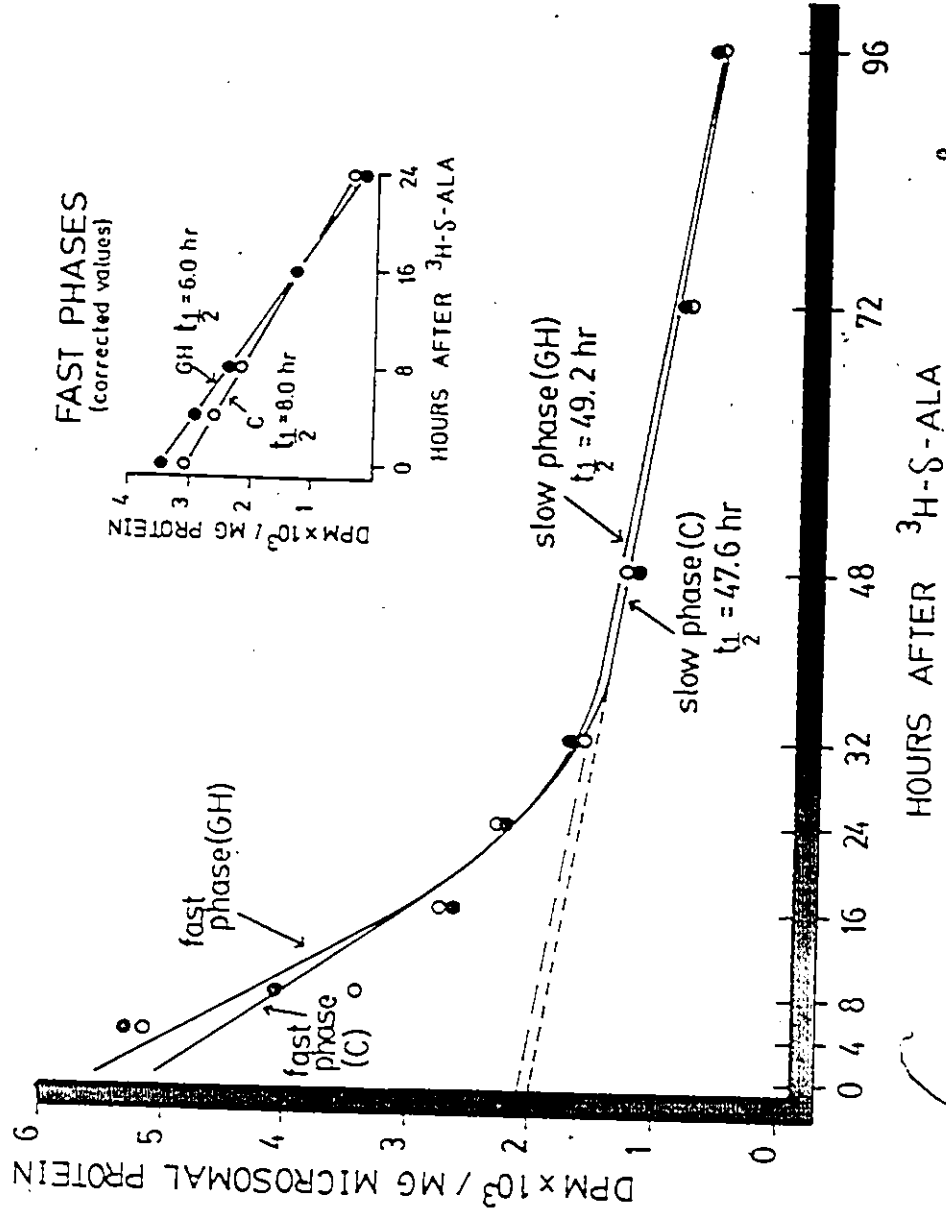
Table XII Effects of growth hormone (10 ug/hr for 5 days) on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats.
Data are the means \pm S.E. of four rats.

Parameter	Growth Hormone (10 ug/hr for 5 days)	Control
Body Wt. (g)	170.8 \pm 4.1	157.0 \pm 4.2
Liver Wt. (mg/g BW)	36.6 \pm 1.3	35.3 \pm 3.3
Microsomal protein (mg/g liver)	15.0 \pm 0.91	15.5 \pm 0.72
EMDM (nmol/mg MP/min)	2.19 \pm 0.26**	4.22 \pm 0.45
ANH (nmol/mg MP/min)	0.594 \pm 0.037	0.661 \pm 0.050
Cytochrome P450 (nmol/mg MP)	0.510 \pm 0.083**	0.933 \pm 0.068
Reductase (nmol/mg MP/min)	45.2 \pm 0.91	44.1 \pm 2.25

* p < 0.05

** p < 0.01

Figure XIII Cytochrome P450 turnover in hepatic microsomes from untreated (control) and GH-treated (10 ug/hr for 5 days, s.c.) hypophysectomized female rats. Animals were injected with 70 uCi/kg of ^3H - γ -ALA (s.a. = 0.8 mCi/nmol) and killed 4-96 hours later. Microsomes were prepared, freed of cytochrome b_5 , and their radioactivity was measured. Each point is the mean of four rats.



removal of GH and other anterior pituitary hormones (Table XIII). Thus, in the hypophysectomized female the total P450 increased by 26% and while there was no change in the size of the FP population, there was a 142% increase in the SP population. Therefore, as in the male, some pituitary factor, possibly GH, is inhibiting the synthesis of the SP-P450 in the intact female. However, in the female, the SP-P450 appears to be depressed to a greater extent than in the male. When the effects of hypophysectomy on EMDM activity and NADPH cytochrome c reductase were examined, the intact female had activity levels of 3.68 and 77.8 nmol/mg MP/min. (Table I) while the hypophysectomized female had values of 3.23 and 36.1 nmol/mg MP/min., respectively (average of all female controls). Thus, removal of the pituitary gland in the female has no effect on ethylmorphine metabolism but does result in a 54% reduction in reductase activity. What is also important to note is that the hypophysectomized female is essentially no different than the hypophysectomized male with respect to EMDM (Figure V), reductase (Figure IV) and the ratio of FP to SP-P450 (Table VI).

The infusion of GH into the hypophysectomized female at a rate of 10 ug/hr for 5 days resulted in significant changes in both FP- and SP-P450 (Table XIV). Both populations were decreased similarly (FP by

Table XIII Effects of hypophysectomy (Hx) on the amount of fast-phase (FP) and slow-phase (SP) hepatic microsomal cytochrome P450 from female rats.
Value in brackets represents percentage of total.

	Cytochrome P450 (nmoles/mg MP)	Relative Amounts FP SP		Ratio of FP/SP fractions
Intact *	0.800 \pm 0.029	0.646 (81%)	0.154 (19%)	4.2:1
Hx **	1.004 \pm 0.080	0.633 (63%)	0.372 (37%)	1.7:1

* Intact female values obtained from Table I

** Hx female values are an average of control values from Tables XIV and XVI

Table XIV Effects of growth hormone (10 ug/hr for 5 days) on the amount of fast-phase (FP) and slow-phase (SP) hepatic microsomal cytochrome P450 from hypophysectomized female rats.
Value in brackets represents percentage of total.

	Cytochrome P450 (nmoles/mg MP)	Relative Amounts FP SP		Ratio of FP/SP fractions
Control	0.933 \pm 0.068	0.564 (60%)	0.369 (40%)	1.5:1
GH	0.510 \pm 0.083	0.319 (63%)	0.191 (37%)	1.7:1

43% and SP by 48%) and thus there were no overall changes in the FP/SP ratio. This GH effect is different from that seen in the males, where feminization was accompanied by a selective decrease in only the SP-P450. In the female, feminization (decrease in EMDM; Figure XI) also occurs, however, we were unable to attribute this to specific decreases in SP-P450 or in FP-P450. In an attempt to clarify the relative roles of the FP and SP populations, this experiment was repeated with a lower dose of GH.

Since a GH dose of 10 ug/hr for 5 days had equal effects on FP and SP-P450, it was decided that a lower GH dose should be tried. In the GH X puromycin experiment (above), 10 ug/hr of GH for 5 days may be yielding almost maximal effects and thus may have caused the interaction effect between GH and puromycin. Therefore, we first performed a preliminary experiment with a GH dose of 7.5 ug/hr for 5 days, to determine its effects on the MFO of the hypophysectomized female. The results of Table XV indicate that the lower GH dose caused less of a decrease in EMDM (17% versus the 48% effected with 10 ug/hr for 5 days; Table XII) and in cytochrome P-450 (28% versus the 45% effected with 10 ug/hr for 5 days; Table XII). Since the cytochrome and the demethylase responded differentially to the two GH doses, it was hypothesized that the FP- and SP-P450s

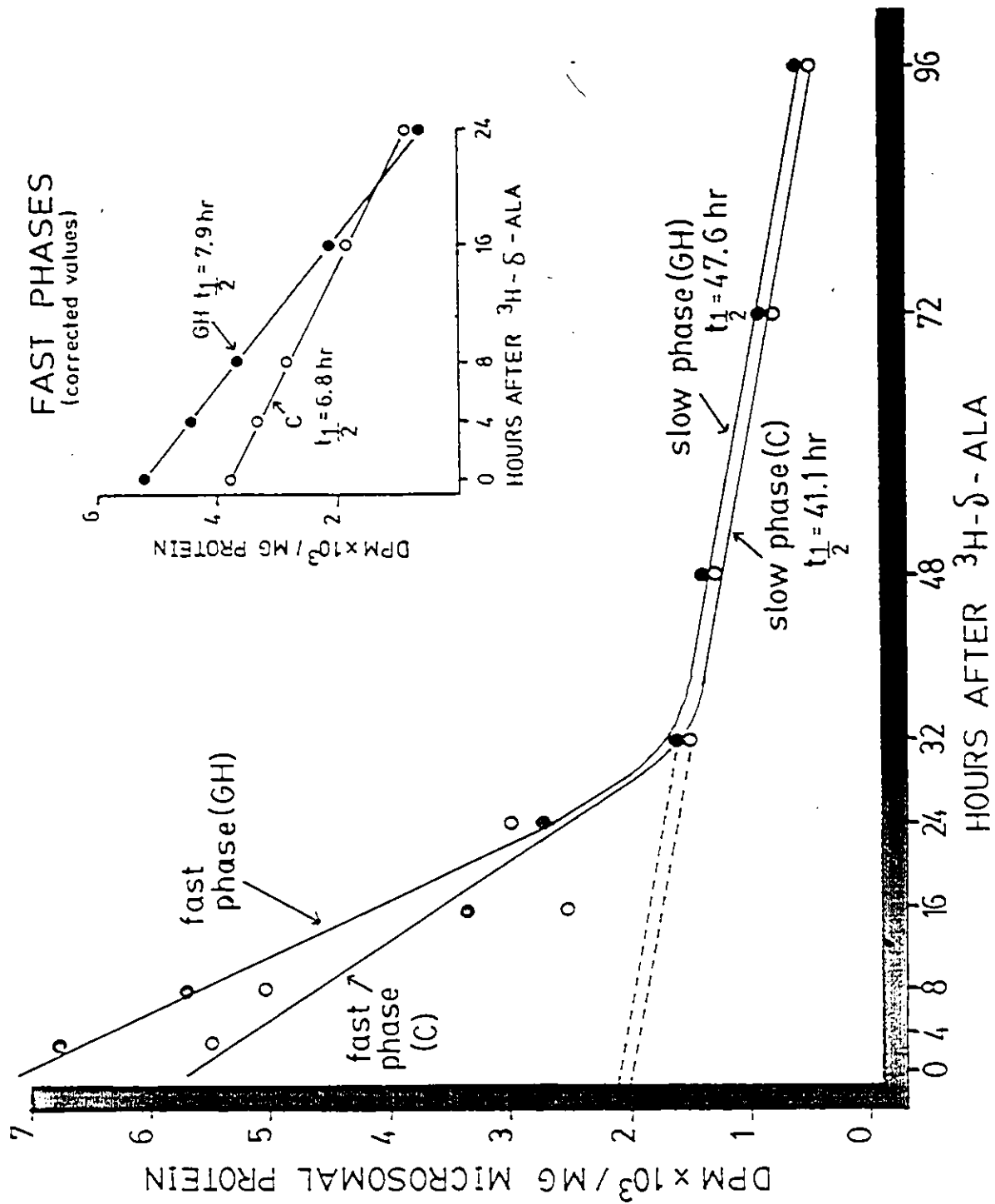
Table XV Effects of growth hormone (7.5 ug/hr for 5 days) on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats.
Data are the means \pm S.E. of four samples.

Parameter	Growth Hormone (7.5 ug/hr for 5 days)	Control
Body Wt. (g)	175.0 \pm 3.9	147.0 \pm 6.9
Liver Wt. (mg/g BW)	34.9 \pm 2.36	33.6 \pm 2.88
Microsomal protein (mg/g liver)	17.8 \pm 1.63	17.6 \pm 2.52
EMDM (nmol/mg MP/min)	2.57 \pm 0.310	3.11 \pm 0.199
ANH (nmol/mg MP/min)	0.549 \pm 0.033*	0.673 \pm 0.035
Cytochrome P450 (nmol/mg MP)	0.776 \pm 0.064*	1.075 \pm 0.093
Reductase (nmol/mg MP/min)	38.3 \pm 3.21	32.7 \pm 1.76

* $p < 0.05$

** $p < 0.02$

Figure XIV Cytochrome P450 turnover in hepatic microsomes from untreated (control) and GH-treated (7.5 ug/hr⁴ for 5 days, s.c.) hypophysectomized female rats. Animals were injected with 70 uCi/kg of ³H- δ -ALA (s.a.=0.8 mCi/nmol) and killed 4-96 hours later. Microsomes were prepared, freed of cytochrome b₅, and their radioactivity was measured. Each point is the mean of four rats.



would also vary in their sensitivity to GH.

Therefore, the effects of a GH dose of 7.5 ug/hr for 5 days on P450 turnover was examined in the hypophysectomized female. The results are presented in Figure XIV where it can be seen that there were no significant changes in the half-lives of the two populations with GH treatment. This further supports the hypothesis that the GH-effected decrease in cytochrome P450 is due to a decrease in the synthesis of P450 and not due to an increase in its degradation. The results of Table XVI, however, demonstrate that the FP- and SP-P450s respond differently to a GH dose of 7.5 ug/hr compared to one of 10 ug/hr. While both FP- and SP-P450s were decreased similarly by a GH dose of 10 ug/hr for 5 days (Table XIV), a dose of 7.5 ug/hr for 5 days effected less of a decrease in the FP-P450 (20%) while still maintaining a large decrease in SP-P450 (41%). Therefore, it may be that the SP-P450 is more sensitive to the effects of GH. The changes in P450 effected with this lower GH dose in the female are similar to those seen in the male with a GH dose of 5 ug/hr for 5 days (Table VI), ie., a 56% decrease in SP-P450 with little effect on the FP-P450. Therefore, GH increases the FP/SP ratio (female-type pattern) by suppressing the SP-P450 to a greater extent than the FP.




Table XVI Effects of growth hormone (7.5 ug/hr for 5 days) on the amount of fast-phase (FP) and slow-phase (SP) hepatic microsomal cytochrome P450 in hypophysectomized female rats. Value in brackets represents percentage of total.

	Cytochrome P450 (nmoles/mg MP)	Relative Amounts FP SP		Ratio of FP/SP fractions
Female	1.075 \pm 0.093	0.701 (65.2%)	0.374 (34.8%)	1.9:1
GH	0.776 \pm 0.064	0.556 (71.6%)	0.220 (28.4%)	2.5:1

In order to determine which P450 population was responsible for the decrease in EMDM, we must compare the effects of the two GH doses (7.5 and 10 ug/hr for 5 days) on the amounts of SP- and FP-P450 and on the rates of ethylmorphine metabolism. Thus, at 7.5 ug/hr GH did not significantly reduce EMDM activity but at 10 ug/hr it decreased it by 48% ($p < 0.01$), that is, feminization resulted. However, although the increase in GH caused feminization, the reduction in SP-P450 was the same at the two doses. Thus, the decrease in EMDM activity is clearly not due to the decrease in the SP population. However, at 10 ug/hr, for 5 days, GH caused a greater decrease in FP-P450 (43%) than it did at 7.5 ug/hr which only slightly affected (20% decrease) the FP-P450. Therefore, feminization of EMDM in the hypophysectomized female appears to be due to a decrease in the FP population of P450 and not to a reduction in SP, as is the case in the male.

(iii) Apocytochrome P450 Content in Hepatic
Microsomes from GH-treated Hypophysectomized
Female Rats

The GH-induced decrease in cytochrome P450 could be caused by either a decrease in apocytochrome P450 synthesis or in heme synthesis. Therefore, microsomes were prepared from GH-treated and control females and aliquots were incubated in the presence and absence of

hemin. It was found that incubation with hemin did not increase the amount of detectable cytochrome P450 in either controls or GH animals, thus showing that essentially all apocytochrome P450 was bound to heme (Figure XV). Therefore, the decrease in P450 is due to a decrease in synthesis of apoenzyme and not heme.

(iv) Puromycin and the Selective Depletion of Fast-phase (FP) Cytochrome P450.

In an effort to clarify the role of FP- and SP-P450s in the MFO system, we attempted to specifically depress the FP-P450 population with puromycin in order to follow the subsequent changes in the MFO parameters that might reveal the function of the FP-cytochrome P450(s). It was hypothesized that two injections of puromycin within 24 hours would markedly decrease the FP-P450s (half-life about 7 hours) while the SP-P450s (half-life about 48 hours) would be only minimally affected. This was not the case, however. Experiments with 75 and 100 mg/kg of puromycin failed to cause any decline in cytochrome P450 levels (Table XVII). Therefore, further work in this area was not pursued.

D. Intact Females

(i) Growth hormone: Effect on the Hepatic MFO

In the previous experiments with hypophysectomized

Figure XV Effect of growth hormone (10 ug/hr for 5 days) on hepatic microsomal apocytochrome P450 content of hypophysectomized female rats. Microsomes were incubated with and without hemin and examined for an increase in cytochrome P450 levels. Data are the means + S.E. of three rats.

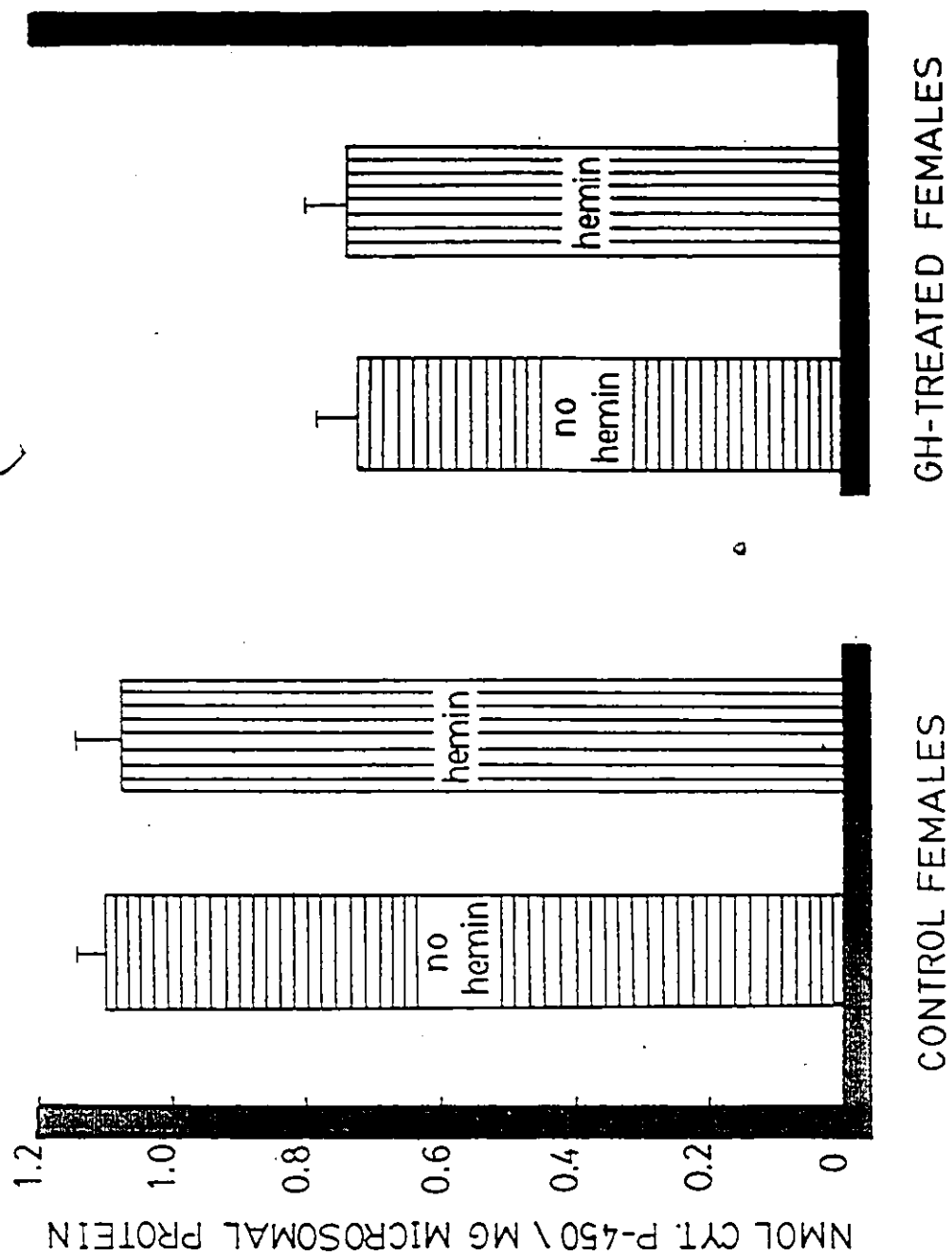


Table XVII The effects of puromycin (75 and 100 mg/kg, twice within 24 hours) on the hepatic microsomal mixed function oxidase system from hypophysectomized female rats. Data are the means \pm S.E. of three rats.

Parameter	Puromycin		Controls
	(75 mg/kg)	(100 mg/kg)	
Body Wt. (g)	153 \pm 2.5	150 \pm 0.5	168
Liver Wt. (mg/g BW)	29.2 \pm 1.15	35.0 \pm 1.65	41.1
Microsomal protein (mg/g liver)	16.0 \pm 0.75	16.0 \pm 0.40	14.2
Cytosolic protein (mg/ml)	25.8 \pm 1.86	21.3 \pm 0.65	25.9
Plasma protein (mg/ml)	41.7 \pm 1.30	34.2 \pm 2.06	56.4
EMDM (nmol/mg MP/min)	3.40 \pm 0.61	3.74 \pm 0.17	3.20
ANH (nmol/mg MP/min)	0.504 \pm 0.116	0.592 \pm 0.016	0.484
Cytochrome P450 (nmol/mg MP)	0.857 \pm 0.132	1.070 \pm 0.025	0.912
Reductase (nmol/mg MP/min)	42.7 \pm 3.81	50.5 \pm 4.71	42.1

female rats, it was found that a GH dose of 10 ug/hr for 5 days would cause a decrease in EMDM activity and in cytochrome P450 levels, an effect termed feminization. We, therefore, determined if a similar dose of GH would feminized the MFO of intact females. The results are shown in Table XVIII. Growth hormone significantly increased body weight ($p < 0.01$) and decreased aniline hydroxylase ($p < 0.05$); however, it had no significant effect on EMDM or on cytochrome P450. These data suggest that the intact female may not respond because it is already responding maximally to endogenous GH. Because the hypophysectomized female has no circulating GH, it is able to respond to the effects of exogenous, infused GH.

(ii) Methyltrienolone: Effect on the Hepatic MFO

No feminizing effects were found when GH was infused into the intact female. Next, we examined the effects of methyltrienolone on the MFO in intact females. Methyltrienolone, is a potent synthetic androgen which is known to operate at the level of the hypothalamus and thus might interfere with GH release from the anterior pituitary gland. Treated rats were injected daily for 14 days with methyltrienolone (125 ug/ day) while controls received the vehicle only. The effects of this treatment on the MFO is summarized in

Table XVIII Effects of growth hormone (10 ug/hr for 5 days) on the hepatic microsomal mixed function oxidase system from intact female rats. Data are the means \pm S.E. of six rats.

Parameter	Growth Hormone (10 ug/hr for 5 days)	Control
Body Wt. (g)	173.0 \pm 1.2**	161.5 \pm 3.0
Liver Wt. (mg/g BW)	47.3 \pm 1.0	48.4 \pm 1.1
Microsomal protein (mg/g liver)	17.5 \pm 0.8	18.2 \pm 0.4
EMDM (nmol/mg MP/min)	2.34 \pm 0.10	2.81 \pm 0.19
ANH (nmol/mg MP/min)	0.612 \pm 0.017*	0.750 \pm 0.037
Cytochrome P450 (nmol/mg MP)	0.739 \pm 0.019	0.764 \pm 0.021
Reductase (nmol/mg MP/min)	105.6 \pm 5.2	112.4 \pm 7.8

* p < 0.02

** p < 0.01

Table XIX

Effect of methyltrienolone (125 ug/day, for 14 days) on the hepatic microsomal mixed function oxidase system from intact female rats.
Data are the means \pm S.E. of seven rats for the treated animals and four rats for the controls.

Parameter	Methyltrienolone (125 ug/day)	Control
Body Wt. (g)	177.6 \pm 2.4*	169.3 \pm 0.75
Liver Wt. (mg/g BW)	47.4 \pm 1.62	45.8 \pm 2.08
Microsomal protein (mg/g liver)	18.3 \pm 0.87	20.1 \pm 0.99
EMDM (nmol/mg MP/min)	5.68 \pm 0.70*	3.47 \pm 0.18
ANH (nmol/mg MP/min)	1.000 \pm 0.060	0.818 \pm 0.022
Cytochrome P450 (nmol/mg MP)	0.803 \pm 0.032	0.718 \pm 0.023
Reductase (nmol/mg MP/min)	123.4 \pm 8.71	111.2 \pm 11.00

* $p < 0.05$

Table XIX. The significant increase in body weight with methyltrienolone treatment was possibly due to the anabolic effects of this potent androgen. As well, methyltrienolone significantly increased EMDM (64%, $p < 0.05$). How this increase in drug metabolism was effected, however, is not clear as both cytochrome P450 and NADPH cytochrome c reductase were unaffected by treatment with the androgen. It is possible that there was an increase in those isozymes responsible for ethylmorphine metabolism at the expense of other P450 isozymes, and thus no overall change in total P450 was apparent.

Summary of Results

1. Intact Animals

a) Sex differences were found to exist for ethylmorphine demethylase (EMDM), aniline hydroxylase (ANH) and NADPH cytochrome c reductase with males possessing significantly greater levels of activity than the females.

b) The total amount of cytochrome P450 was not sexually differentiated, ie., males and females had similar quantities.

c) Males possessed more of the SP or slow turnover cytochrome P450 (FP/SP ratio was 2.4:1) while the females had a greater amount of the FP or fast turnover

population of cytochrome P450 (FP/SP ratio was 4.2:1).

2. Hypophysectomized Male

a) Treatment of the hypophysectomized male with GH (5 ug/hr for 5 days) resulted in a 27% decrease in EMDM activity (ie., feminization).

b) GH decreased the amount of SP-P450 by 56% but lowered the FP-P450 by only 17%. Therefore, the decrease in EMDM was attributed to a decrease in the SP-P450 population.

c) The lowering of SP-P450 by GH resulted from a decrease in the synthesis of the apocytochrome, most likely at the translational level. This conclusion was based on results obtained from several experiments:

(i) Since the half-lives of both the FP- and SP-P450 were increased by 80% and 67%, respectively, the decrease in P450 could not be attributed to an increase in degradation. Therefore, the decrease must result from a decrease in synthesis.

(ii) When microsomes from GH-treated males were incubated in the presence of hemin, no increase in P450 resulted. Thus, all the apoenzyme was already bound to heme. It follows that GH decreases the synthesis of apocytochrome P450 and ~~not~~ heme.

(iii) GH exhibited effects qualitatively identical to those of puromycin, a translational inhibitor of protein synthesis and their effects were statistically

additive. Thus GH decreases apoprotein synthesis at translation.

d) GH causes the ratio of FP to SP cytochrome P450 to increase towards the female value. This is due to the SP-P450, which is the more GH-sensitive, being depressed to a greater extent than the FP-P450.

3. Hypophysectomized Female

a) Treatment of the hypophysectomized female with GH (10 ug/hr for 5 days) resulted in a 48% decrease in EMDM activity (ie., feminization).

b) At this dose, GH decreased the SP-P450 by 48% and the FP-P450 by 43%.

c) The reduction in both P450 populations was due to a decreased synthesis of apocytochrome P450. This conclusion was based on these results:

(i) Since the half-lives of both SP- and FP-P450 were unaffected by GH, the decrease in P450 could not be attributed to an increase in degradation. Therefore, it follows that the decrease in P450 resulted from a decrease in synthesis.

(ii) Reconstitution experiments, with hemin revealed no free apoenzyme, that is, heme was saturating all available apoenzyme. Therefore, GH decreases the synthesis of apocytochrome P450 and not heme.

d) The statistically significant interaction between GH and puromycin in decreasing cytochrome P450,

implies that GH does not act exclusively through the same mechanism as puromycin. However, the non-additivity of GH and puromycin effects may be the result of using high doses of these agents. The results, therefore, are ambiguous and it is uncertain as to whether GH acts only at the level of translation or also at sites elsewhere to depress P450 synthesis.

e) GH, at 7.5 ug/hr for 5 days, caused no significant decrease in ethylmorphine metabolism, a 20% decrease in FP-P450 and a 41% decrease in SP-P450. Therefore, the SP-P450, as was the case in the males, is more sensitive to GH.

f) In the female, the decrease in ethylmorphine metabolism can best be attributed to a decrease in FP-P450. Thus:

(i) The population of SP-P450 was decreased similarly (about 45%) by GH, either 7.5 or 10 ug/hr for 5 days.

(ii) GH, at 10 ug/hr, substantially decreased (43%) FP-P450 but had relatively little effect (20% decrease) at 7.5 ug/hr.

(iii) GH, at 10 ug/hr, caused a significant decrease (48%) in EMDM activity; at 7.5 ug/hr the decrease (17%) was not significant.

4. Intact Female

a) The continuous infusion of GH (10 ug/hr for 5

days) into the intact female causes no feminization of the MFO.

b) In the intact female methyltrienolone (125 ug, daily, for 14 days) results in an increase (63%) in EMDM activity with no concomitant effect on total cytochrome P450 or on NADPH cytochrome c reductase activity.

From the results, it is clear that GH is able to feminize the MFO of males and females by decreasing the synthesis of apocytochrome P450. However, the mechanism by which the GH-effected feminization occurs is sexually differentiated. In the male, feminization is due to a decrease in the synthesis of SP-P450 at the translational level. In the female, on the other hand, feminization is due to a decrease in the synthesis of FP-P450 at the translational level or also at sites elsewhere.

DISCUSSION

The mechanism by which GH inhibits or feminizes the hepatic MFO was unknown, prior to this study, as there was no case where feminization could be attributed to a reduction in an enzyme or enzymes. Therefore, the goal of this study was to determine the mechanism of GH action. We find that in hypophysectomized males, feminization of EMDM activity by GH is due to the decreased synthesis of slow-phase (SP)-apocytochrome P450(s). This apparently occurs at the translational level. On the other hand, in hypophysectomized females, GH feminizes by inhibiting the synthesis of fast-phase (FP)-apocytochrome P450(s). The site of GH action in the female may be at the level of translation and/or other events of protein synthesis.

In this discussion, I'll first consider the sex differences that exist, the proposed reason for their existence and the early evidence for the involvement of GH. Then, our results concerning the mechanism of GH feminization of the MFO will be discussed.

Growth Hormone and the Sex Differences in the Rat Hepatic MFO System

Sex differences in the rates of hepatic drug metabolism are known to exist in the rat and have been

extensively studied. In the present study, male rats metabolize ethylmorphine and aniline to a significantly greater extent than do females. While the published data also indicates a 3-4 fold higher activity of ethylmorphine N-demethylase in the male (Davies et al., 1968; Kato, 1974; Colby, 1980), aniline hydroxylase has been found to exhibit either no sex difference (Kato and Gillette, 1965; El Defrawy El Masry et al., 1974) or a small (30-35%), but significant, sex difference (male > female) (this study; Kato, 1974). Such discrepancies have been attributed to rat strain differences, intra-laboratory variation, and other factors. As well, our work reveals that the activity of NADPH cytochrome c reductase is also sexually-differentiated (male > female), however, no differences are apparent in the amounts of total hepatic microsomal cytochrome P450. Although, similar findings have been repeatedly reported (Kramer and Colby, 1976; Colby, 1980; Lui et al., 1982; Virgo, 1983 and 1985), Kato (1974, 1979) finds 20-30% more cytochrome P450 in males.

Sex differences in any system would lead one to suspect the involvement of the gonads and gonadal hormones. Indeed, sex differences in P450-dependent enzyme activities are modulated by two distinct components of androgen action on the hepatic MFO.


Androgens increase the activity of EMDM (Kato, 1974; Kramer et al., 1978a) as well as cytochrome P450 content and NADPH cytochrome c reductase activity (Kato and Onoda, 1970) in castrate males. Therefore, part of the stimulatory effect of testosterone depends on the continuous exposure to androgens in the adult period (Colby, 1980; Skett and Gustafsson, 1980). Castration of adult males results in a decrease in the rates of many P450-dependent reactions and hence partial feminization of the hepatic MFO (Chung, 1977; Colby, 1980; Gustafsson et al., 1983a,c). Complete feminization of the enzyme activities of the male can be achieved by castration of the neonate (Gustafsson et al., 1983a). Therefore, the second component of androgen action involves its effect(s) during early, postnatal life (Einarsson, 1973; Chung, 1977). Neonatal exposure to androgens imprints or programmes basal activities and sensitizes enzymes to further activation by testosterone in the adult (Colby, 1980; Chung and Chao, 1980; Mode and Norstedt, 1982; Gustafsson et al., 1983a,c).

Testicular androgens cause the development of sex-dependent forms of hepatic cytochrome P450 which may account, at least in part, for the sex differences in hepatic drug and steroid metabolism (Chao and Chung, 1982; Chung et al., 1981; Kamataki et al., 1983, 1985;

Waxman et al., 1985). Rat liver contains at least nine structurally distinct and catalytically different forms of microsomal P450 (Guengerich et al., 1982; Dannan et al., 1983; Ryan et al., 1984; Waxman et al., 1985), three of which are constitutive, sex-dependent forms—two male-specific and one female-specific. One of the male-specific isozymes is responsible for about 85% of the steroid 16 α -hydroxylase activity present in uninduced, mature, male rat liver (Waxman, 1984). This form also has a high turnover number for various drugs, including ethylmorphine (Morgan et al., 1985). Because several different laboratories have isolated and purified this isozyme (Kamataki et al., 1984; Ryan et al., 1984; Waxman et al., 1985; Morgan et al., 1985), it has been designated by several names; however, in this discussion it will be referred to as P450h (Ryan et al., 1984). The other male-specific P450 is termed P450-PB-2a/PCN-E (Waxman et al., 1985) or P450g (Ryan et al., 1984) and mediates the majority of microsomal steroid 6 β -hydroxylase activity. As well, it metabolizes aminopyrine, ethylmorphine and benzapyrene but at a slower rate than P450h (Cheng and Schenkman, 1982). The female-specific isozyme, P450i, which has recently been characterized by MacGeoch and co-workers (1984) is most active in the 15 β -hydroxylation of steroid sulfates. It also metabolizes a variety of

structurally-diverse drug substrates and exhibits lower catalytic activity than P450h with most, but not all, of these compounds (Waxman, 1984; Waxman et al., 1985). There is evidence that P450h is imprinted by neonatally-released testicular steroids. As well, neonatal androgen is responsible for maintaining relatively high levels of P450g in maturing males and for the decrease in P450i that occurs in male rats after 4 weeks of age (Waxman et al., 1985). Therefore, the presence of P450h in the adult male, compared to P450i in the female, may partially explain the preferential metabolism of many xenobiotics by male rat liver.

Results from this study also confirm the presence of sex differences in hepatic P450. We found that both adult male and female rats exhibit a biphasic turnover of hepatic microsomal cytochrome P450, thus demonstrating the existence of two populations of P450—a fast phase (FP) population, with a half-life of about 6 hours, and slow phase (SP) population, with a 34-hour half-life. Other investigators (Garner and McLean, 1969; Levin and Kuntzman, 1969; Levin and Ryan, 1975; Lui et al., 1982) have reported similar P450 turnover dynamics with half-lives (5-8 hours and 40-44 hours) comparable to those obtained in our study. Our work also reveals that while the total microsomal P450



content is similar in the two sexes, the levels of SP-P450 are greater (38%) in males while the levels of FP-P450 are greater (25%) in females. These observations are consistent with the findings of Levin and Ryan (1975) and Lui and associates (1982) except they found no sex differences in the amounts of the FP-P450 population. This may simply be the result of the females in our study having slightly more total P450 than the males rather than less total P450, as was the case in the experiments of others. However, in all studies, there are clearly sex differences in the two P450 populations which may cause or contribute to the sexual dimorphism in hepatic drug metabolism.

The ratio of FP- and SP-P450, in addition to being sex-dependent, is also neonatally imprinted. Both adult males and males castrated at 4 weeks (ie., post-neonatal imprinting) have a greater amount of SP-P450 than do rats not neonatally imprinted, such as females and adult males castrated at birth (Levin and Ryan, 1975). Since the imprinting of the male-specific isozymes, P450g and P450h, coincides with the imprinting of the SP-P450 population, it may be that P450h and g are part of the slow turnover population. Further support for this suggestion is provided by the observations of Chao and Chung (1982), who found that the neonatally androgen-imprintable testosterone 16 -

hydroxylase (most likely P450h) has a slow rate of turnover (half-life > 48 hours). Still others (Shiraki and Guengerich, 1984) have reported a half-life of only 20 hours for P450h. The lack of androgen imprinting in the female rat, however, allows for the developmental induction of P450i, which reaches its adult level by 8 weeks of age (Waxman et al., 1985). Since our studies revealed a greater amount of FP-P450 in the female, this may suggest that P450i is an isozyme within the fast turnover population. There is, however, no direct evidence to support this possibility.

The effects of estradiol on the hepatic MFO of the female rat are much less defined than the actions of androgens in the male. Ovariectomy of the female rat, neonatally or postpubertally, has little effect on drug metabolism including EMDM (Colby, 1980) which would suggest that estradiol is not necessary to maintain normal levels of enzymes in the female. However, when estradiol is administered to normal or castrated males, there is a decrease in drug metabolism and P450 levels, hence a shift towards a female-type of metabolism (Kramer et al., 1978a; Gustafsson et al., 1980; Mode and Norstedt, 1982). It has also been shown that the continuous presence of estradiol in the male will reverse neonatal imprinting (Gustafsson et al., 1983c).

Although, testosterone and estradiol exert

opposite effects on the hepatic MFO of males, they both require an intact pituitary gland for manifestation of their effects (Gustafsson and Stenberg, 1976; Kramer et al., 1978b; Colby, 1980). The fact that the establishment of sex differences coincides with maturation of the pituitary gland and that androgens and estrogens are known to influence pituitary hormone secretion (Gustafsson et al., 1983b,c), indicates that sex steroids act on the liver through an effect on the pituitary gland and not a direct effect on the liver itself. The results of this study show that removal of the pituitary gland abolishes the sexual differentiation of EMDM and NADPH cytochrome c reductase that exists in intact animals. These data are consistent with those of Gustafsson et al. (1980) who found that EMDM activity is pituitary-dependent and hypophysectomy abolishes the sex differences in hepatic drug and steroid metabolism. As well, our work also demonstrates that the turnover of P450 is controlled by the pituitary gland. Hypophysectomy of either sex results in a significant increase in the SP-P450 population but has no effect on the FP-P450. The fact that this increase in SP-P450 is not accompanied by an increase in drug metabolism is probably due to the accompanying 50% reduction in NADPH cytochrome c reductase, the rate-limiting enzyme in P450-mediated

reactions (Matsuura et al., 1978; Nisimoto et al., 1983). Lui and co-workers (1982) also found a preferential increase in the concentration of the SP-P450 population with hypophysectomy and suggested that the sex difference in the MFO of adult rats is associated with the SP hemoprotein(s). The involvement of the pituitary gland in hepatic drug metabolism is still further implicated by the finding that implantation of a pituitary gland under the kidney capsule of adult hypophysectomized rats results in feminization of the MFO (Gustafsson and Skett, 1978; Colby, 1980).

These data indicate that the pituitary gland secretes a "feminizing factor" (Gustafsson and Stenberg, 1976; Gustafsson and Skett, 1978) responsible for feminization of drug metabolism and possibly for the suppression of the SP-P450(s). Experiments with pituitary hormones and drugs that affect their release in vivo, reveal that only growth hormone (GH) is able to feminize, that is, decrease drug metabolism in intact, castrate or hypophysectomized male rats (Wilson and Spelsberg, 1976; Kramer and Colby, 1976; Kramer et al., 1978a; Mode et al., 1981, 1983; Virgo, 1983, 1985). Decreases in both P450 content (Kramer et al., 1978a) and reductase (Wilson, 1973; Virgo, 1985) have been found to accompany this feminization of the MFO. In

this study, we also find that GH decreases EMDM activity and cytochrome P450 levels in hypophysectomized male and female rats. However, the dose of GH required to achieve feminization in the hypophysectomized female was twice that needed in the males, indicating that there is a sex difference in GH responsiveness of the male and female hepatic MFO or that there are sex differences in the pharmacokinetics of GH. On the other hand, GH treatment of the intact female rat effects no significant reductions in either P450 or EMDM (this study; Kramer et al., 1975, 1978a; Virgo, 1985). Since GH affects hypophysectomized females, our results suggest that endogenous GH is already feminizing the female MFO maximally.

GH purified from either male or female rats is equally potent in feminizing liver metabolism (Mode et al., 1983). Moreover, there are no sex differences in the mean plasma levels of GH (Jansson et al., 1984). This tends to argue against the possibilities of a sex-specific form of GH or the release of more GH, either of which could feminize the female hepatic MFO. What is sexually-differentiated, however, is the secretory pattern of GH. Male animals have regular surges of GH every 3-4 hours that results in plasma levels of 400-500 ng/ml, with low or undetectable levels between the peaks, whereas females have irregular bursts of GH with

lower peak levels and higher interpeak levels than the male (Eden, 1979; Mode et al., 1982; Jansson et al., 1984, 1985a). Some have suggested that it is this sex difference in GH release that may be responsible for the sex differences in hepatic drug and steroid metabolism (Mode et al., 1981, 1982; Gustafsson et al., 1983c). When GH (120 ug/day) is administered to the hypophysectomized female with two subcutaneous injections daily, no feminization results, however, infusion or frequent administration results in a progressive increase in the degree of feminization (Mode et al., 1982; Gustafsson et al., 1983b). But the fact that one is also able to feminize with two injections/day of a high enough dose of GH suggests that both dose and administration of GH are critical determinants for feminization. In our experiments, we infused GH continuously via osmotic minipumps as this should result in blood levels of GH which are characteristic of the female.

Further work in this area has revealed that it is the sex steroids that influence this plasma pattern of GH in male and female rats. Testosterone treatment of neonatally castrated rats results in "masculinization" (ie., increase in drug metabolism) of the MFO and is accompanied by an increase in maximum and a decrease in minimum GH levels (Mode et al., 1982; Gustafsson et

al., 1983b; Jansson et al., 1985a,b). Castration or estradiol treatment of the male rat, regimes which cause feminization of the hepatic MFO, are associated with a decrease in maximum and an increase in minimum GH levels (Gustafsson et al., 1983b; Jansson et al., 1985a). Therefore, the continuous presence of detectable GH may be a prerequisite for feminization. In adult males, the exposure to neonatally-secreted testicular androgens imprints the high amplitude pulses characteristic of GH secretion in this sex and simultaneously masculinizes the pattern of hepatic drug and steroid-metabolizing enzymes (Jansson et al., 1985a).

Sex steroids affect the release of GH, and hence modulate enzyme patterns in the hepatic MFO, via control at the level of the hypothalamus (Gustafsson et al., 1979, 1980, 1983b and c). There are two hypothalamic factors that are responsible for controlling the release of GH from the anterior pituitary gland: somatostatin (SS), which inhibits release, and growth hormone releasing factor (GRF) (Jansson et al., 1985b). Castration, anterior hypothalamic deafferentation and neutralization of SS with anti-SS antiserum all decrease the hypothalamic content of SS in the male rat and thus increase basal plasma levels of GH and feminize the MFO (Gross, 1980;


Colby, 1980; Gustafsson et al., 1980, 1983a,b; Jansson et al., 1985b). It has been proposed that neonatal androgens imprint higher levels of SS in the male which consequently more greatly inhibits the release of GH from the pituitary (Gustafsson et al., 1983b). Virgo (1985) has suggested that testosterone influences hepatic drug metabolism in the male rat either by decreasing GH levels or by suppressing the action of GH on the liver. In this study, we find that the administration of the potent synthetic androgen, methyltrienolone, to normal female rats results in an increase in ethylmorphine metabolism (ie., "masculinization"). Therefore, the possibility exists that androgens may be operating at the hypothalamo-pituitary level, lowering GH levels and masculinizing drug metabolism. Moreover, while testosterone increases SS levels in the median eminence and promotes storage of GH in the anterior pituitary gland, estrogens exert opposite effects- decrease SS levels and inhibit storage of pituitary GH (Gross, 1980). This may, therefore, be one explanation for the more or less constant release of GH in female rats. Gustafsson and co-workers (1983c) have further suggested that estradiol may even exert direct action on the pituitary gland itself to stimulate secretion of GH. The presence of high concentrations of both androgen

and estrogen receptors in areas involved in regulation of GH secretion (ie., anterior hypothalamic periventricular area, amygdala, preoptic area) further supports the idea of hypothalamic control of GH release by sex steroids (Jansson et al., 1985b).

While extensive work is being directed towards a better understanding of the hypothalamic imprinting of the MFO by neonatally-secreted testicular androgens, not much research has been conducted regarding the mechanism of MFO feminization by GH. Therefore, this study was carried out primarily to determine the mechanism by which GH feminizes the MFO.

Mechanism of GH action on the Hepatic MFO of the Rat

First, let us consider the mode of action in the male rat. Studies with intact (Wilson, 1973), castrated (Kramer et al., 1978) and hypophysectomized/adrenalectomized male rats (Wilson and Spelsberg, 1976) showed that GH-effected feminization of EMDM activity was accompanied by a decrease in both cytochrome P450 levels and NADPH cytochrome c reductase. Our experiments with hypophysectomized male rats show that GH decreases the hepatic metabolism of ethylmorphine through a decrease in P450 as the reductase was unaffected. All of these data conflict with those of Rumbaugh and Colby (1980) who found that



in the hypophysectomized male, GH increased EMDM but had no effect on P450 (data on reductase levels was not included). Their studies suggested that GH is only able to feminize in the presence of adrenocorticotrophic hormone and thyroid hormone. Since these findings have not been repeated by other researchers, their value is dubious. We and others (Mode et al., 1981, 1982) have feminized hepatic drug and steroid metabolism, respectively, in hypophysectomized male rats with continuous infusion of GH in the absence of other hormones.

In order to compare the amount of plasma GH that results from the pump infusate in the hypophysectomized male, with the concentration of endogenous GH normally present in the intact male, the following equation was used to estimate the concentration of the exogenous GH in the plasma (Mayer et al., 1980):

$$C = \frac{1.44 \times t_{1/2} \times f \times D}{V_d \times T}$$

where C = plateau concentration of GH in plasma (ng/ml); V_d = volume of distribution [20% of average BW (170g) = 34 ml]; $t_{1/2}$ = 0.33 hr. for GH (Murad and Haynes, 1980); D (dose)/ T (dose interval) = infusion rate (5 ug/hr) and f = 1 assuming instantaneous and

100% absorption. Thus, infused GH will plateau at a plasma concentration of 70 ng/ml which is hypophysiologic compared to the mean endogenous GH level in both intact males (253 ng/ml) and females (135 ng/ml) (Jansson et al., 1985a). However, this concentration is hyperphysiologic compared to the minimum plasma level of GH (9 ng/ml) normally present between the GH peaks or surges every 3-4 hours in male rats. This suggests that the continuous presence of hyperphysiologic levels of GH is sufficient for feminization. These results are consistent with the findings of Gustafsson et al., (1983b) who concluded that the absence of time periods with undetectable plasma levels of GH appears to be a major determinant for feminization.

In the present study, we show that GH feminizes EMDM activity in the male rat by inhibiting the synthesis of microsomal cytochrome P450. The involvement of reductase was ruled out as its levels were unaffected by GH treatment. Critical evidence for the GH-effected decrease in P450 synthesis comes from the finding that GH substantially increases the half-lives of both P450 populations- FP and SP. Since the levels of proteins present in cells depends on both synthesis and degradation, the lack of any increase in the rates of degradation (decreased half-life) would

suggest the alternate cause as the reason for the decrease in P450 levels, that is, decreased synthesis. This observation is consistent with that of Shiraki and Guengerich (1984) who found that when the steady-state level of P450 is altered (with phenobarbital), it is primarily due to changes in the rate of synthesis as opposed to changes in the rate of degradation. Degradation of P450, according to these workers, does not appear to be a highly specific process. Furthermore, we find that a decrease in the synthesis of the P450 apoprotein, rather than a decrease in heme synthesis, causes the ultimate reduction in total P450 levels. The apparent lack of a heme deficiency is not surprising as heme is, in most instances, not rate-limiting in P450 synthesis (Bhat et al., 1977). Moreover, the fact that GH affects EMDM but has no effect on ANH further argues against a general decrease in heme levels, since such a decrease should affect both P450-mediated reactions similarly and not differentially.

With the use of puromycin, we were able to determine the site of action of GH in the male, namely, translation. Early studies by Korner (1968, 1969) show that GH actually stimulates the synthesis of most hepatic proteins. That GH stimulates the synthesis relatively rapidly and affects synthesis even when

precursor incorporation into RNA has been inhibited by actinomycin D, is evidence that GH controls protein synthesis at translation (Korner, 1968). Moreover, GH stimulates the ability of the liver microsomes to attach themselves to and translate mRNA (Kostyo and Nutting, 1974). We present, in this study, the first direct evidence that GH also inhibits protein synthesis at the translational locus. In addition, GH not only decreases the amount of cytochrome P450 but also other enzymes such as tryptophan pyrrolase, tyrosine transaminase and serine dehydratase (Wilson, 1973).

While GH does decrease total P450 content in the hepatic microsomes of the hypophysectomized male rat, the decrease does not affect both P450 populations similarly. Rather, we find that there is also a qualitative change with a selective decrease in the SP population. This, together with the fact that the FP-P450 is minimally affected, suggests that the FP- and SP-P450s are regulated either simply by different doses of GH effecting the same mechanism or through different mechanisms. The data of others indicates that GH affects the male- and female-specific isozymes differentially. Morgan et al., (1985a,b) have determined immunologically that GH decreases the amount of P450h in male rats. Since this isozyme exhibits a high catalytic activity towards ethylmorphine (Morgan

et al., 1985a) any reduction in its amount would consequently result in a decrease in ethylmorphine metabolism and feminization of this function. Because GH decreases both P450h (Morgan et al., 1985a,b) and SP-P450 (this study), this may support the possibility that P450h is a SP-P450. However, the only definite conclusion that can be drawn from our data is that inhibition of SP-P450 synthesis by GH causes feminization of EMDM activity in the hypophysectomized male rat.

The effects of GH on the normally low levels of the female-specific isozyme, P450i, in the male have been reported to be the opposite of those on P450h (MacGeoch et al., 1985). GH infusion increases the amount of immunoreactive P450i in both intact and hypophysectomized male rats. Since the turnover number for P450i with ethylmorphine is about 67% that for P450h (Morgan et al., 1985a; MacGeoch et al., 1984), a putative increase in P450i should cause an increase in EMDM activity. However, the large decrease in the SP-P450 population which is seen in the hypophysectomized male would mask any stimulatory effect P450i may exert on EMDM, and thus ethylmorphine metabolism is still significantly decreased.

Our data, therefore, show that the GH-sensitive cytochrome P450 that metabolizes ethylmorphine in the

male is the SP-P450. Although, the identity of the specific isozyme(s) involved is unknown, several possibilities exist. It may be P450h, as mentioned above, or possibly the other male-specific isozyme, P450g, which also metabolizes ethylmorphine. Although, there is no direct evidence to indicate that P450g is decreased by GH, as is P450h, GH does decrease the activity of androgen 6 β -hydroxylase (Mode et al., 1981; Gustafsson et al., 1983a)- a P450g mediated reaction. Yet another possibility is that other, unidentified P450s are involved in ethylmorphine metabolism. Waxman (1984) has pointed out that since P450h and P450i both metabolize ethylmorphine at high rates, these two sex-specific isozymes alone cannot explain the sex difference in EMDM. Therefore, it is probable that other sex-specific P450s also contribute to the sexually-differentiated activity of EMDM.

Thus far we have been discussing the male rat and how GH may be feminizing the MFO in this sex. We will now turn our attentions to the female rat. Our studies on the female rat demonstrate that GH feminizes drug metabolism, as in the male, by decreasing the synthesis of apocytochrome P450. There are, however, several differences.

We find that in order to achieve feminization of EMDM activity, females require double the dose of GH

effective in the male. These results conflict with those of Mode and co-workers (1981), who report that 5 ug/hr of human GH for 7 days feminizes liver steroid metabolism in both hypophysectomized-gonadectomized male and female rats. This difference may be due to either the endpoint that they measure or the fact that they use human GH which is more potent than the ovine GH used in our study.

In order to calculate the plasma level of GH which feminizes the hepatic MFO of hypophysectomized females and compare it with the endogenous levels of GH present in the normal, intact female, we used the equation described by Mayer and associates (1980) on page 122 of this discussion. Infusion rates of 7.5 ug/hr and 10 ug/hr both generate plasma concentrations of GH (105 ng/ml and 140 ng/ml, respectively) substantially higher than the minimum GH levels (32 ng/ml) found in intact females (Jansson et al., 1985a) but only the highest infusion rate, for 5 days, is able to feminize EMDM activity. Therefore, hypophysectomized females require plasma GH levels that are 3-4 fold greater than the basal GH levels which feminize the normal, intact female.

The site at which GH operates to decrease P450 synthesis in the female is unclear as there can be two different explanations for the results obtained in our

study, that is, the interaction effect between GH and puromycin in decreasing P450 levels. Statistically, the data suggest that GH may be operating at additional sites, other than translation, to fully effect feminization in the female. Wilson and Spelsberg (1976) have suggested that GH alters transcription in the nucleus by increasing RNA polymerase II activities and de-repressing chromatin DNA in hypophysectomized/adrenalectomized rats. However, it is also possible that the GH and puromycin dosages employed (10 ug/hr for 7 days and 37 mg/kg, daily, for 5 days) cause maximal effects and therefore mask any additive effect that may exist between GH and puromycin. If this is the case, then GH may indeed act at translation as it does in the male. The 31% decrease in P450 effected with either GH or puromycin and the lack of any further reduction after treatment with both, may support such a possibility.

In addition to the differences mentioned thus far between GH-effected feminization in male and female rats (ie., dose of GH and site of GH action), the female also displays different qualitative changes in P450. At a GH dose of 7.5 ug/hr, there is a selective decrease in the SP-P450, as in the male, and no feminization of EMDM. At 10 ug/hr, however, when both SP- and FP-P450 are substantially reduced, feminization

of EMDM occurs. Therefore, the data leads us to the conclusion that the decrease in EMDM activity in the female is the result of a decrease in FP-P450. From these results, we may also infer that the SP population is more sensitive to the effects of GH and this may be one explanation for females possessing less SP-P450 isozymes. The higher basal levels of GH which exist in the female (Mode et al., 1982; Jansson et al., 1984, 1985a) may suppress SP-P450 to a greater extent than in the male.


Feminization in the male is due to a decrease in the synthesis of SP-P450. Male-specific SP isozymes, if they exist, would probably exist only at very low levels in the female. Therefore, reductions in the SP-P450 population would have no real effect on EMDM activity in the female. Instead, the female appears to possess isozymes which metabolize ethylmorphine and which are part of the fast turnover population of P450. A GH dose of 10 ug/hr decreases these FP isozymes and consequently feminizes EMDM. We can, however, not rule out the possibility that there are also SP-P450 isozymes which metabolize ethylmorphine in the female. Hypophysectomy of the female rat caused a 142% increase in SP-P450 and if NADPH cytochrome c reductase is rate-limiting in this instance (Matsuura et al., 1978), then no increase in EMDM activity would be seen. Similarly,

a 45% decrease (with GH) in these elevated SP-P450 levels would not lower EMDM activity, yet may still decrease SP isozymes that metabolize ethylmorphine.

Now arises the question of how GH acts at the hepatic level. Growth hormone must feminize the hepatic MFO in both male and female rats via a direct effect on the liver. Growth hormone, a polypeptide hormone, binds to the plasma membrane of rat hepatocytes via two different receptors: somatogenic which bind only the purely growth-promoting hormones (eg. rat GH, bovine GH) and lactogenic receptors which also bind lactogenic hormones such as prolactin and human GH (hGH) (Ranke et al., 1976). This binding of GH is known to initiate three different events: generation of a biologic signal, ligand degradation and regulated receptor loss (Hizuka et al., 1981). Once internalized, the GH-receptor complex becomes sequentially associated with plasma membranes and Golgi fractions followed by either a recycling of the hormone-receptor complex (by exocytosis) to the plasma membrane or the lysosomal degradation of GH and receptor (Postel-Vinay et al., 1982). The internalization of the hormone-receptor complex (ie., loss of plasma membrane receptors) stimulates the production of new receptors which are synthesized on the rough ER, concentrated in the Golgi and transferred

via Golgi vesicles to the plasma membrane (Posner et al., 1981). Depending on which process dominates, receptor loss or receptor synthesis, there will either be an increase or decrease in tissue receptor levels and hence either increased or decreased potential for GH binding, respectively. Although, it has been suggested that GH may act through an increase in guanylate cyclase activity (Hadley, 1984), the possibility also exists that GH, or a modified GH product, can act intracellularly to affect translation of P450.

Since both human GH, which has somatogenic and lactogenic properties, and rat GH, which has only somatogenic properties, feminize the MFO, it would appear that changes in P450 synthesis are mediated only by the somatogenic receptors (Gustafsson et al., 1980; MacGeoch et al., 1985). But the fact that males and females have equal numbers of somatogenic receptors with the same affinity for GH (Ranke et al., 1976) cannot explain the sex differences in hepatic drug metabolism. Therefore, discrimination is not at the level of the receptor. It is more likely that the concentration of GH is the determining factor for the sex difference. Since females maintain higher GH levels, then more receptors are occupied in the female more of the time and this may effect feminization in this sex. However, the efficacy of rGH is



substantially less than that of hGH. The simplest explanation for this would be differences in the binding affinity of rGH and hGH for somatogenic receptors. However, it is also possible that the direct action of GH on the liver may be potentiated by additional pituitary factors (possibly prolactin) which may then permit GH to exert its full feminizing effect (MacGeoch, 1985). Since GH induces lactogenic receptors (Baxter and Zaltsman, 1984; Norstedt et al., 1984) and females possess predominantly lactogenic sites (Herington, 1982), this suggestion is possible. Still other investigators (Hughes and Friesen, 1985) have postulated that the different actions of GH are mediated by different "second messengers" or even through yet unidentified receptors.

It is interesting that the effects of GH closely resemble those produced by estradiol. While hypophysectomy and ovariectomy have no real effect in the female, both GH and estradiol do feminize liver enzymes in the male (Colby, 1980). This reduction in hepatic drug metabolism associated with estradiol, as with GH, is directly related to a decrease in components of the microsomal MFO (Kramer et al., 1978; Mackinnon et al., 1977). Mackinnon and co-workers (1977) have found that estradiol decreases the hepatic content of P450 by decreasing the synthesis of

apocytochrome P450 (at least selective apoproteins). Since GH appears to be the hormone which mediates the actions of estradiol on hepatic drug and steroid metabolism (Kramer and Colby, 1976; Rumbaugh and Colby, 1980), this provides indirect evidence that GH inhibits the synthesis of selected apocytochrome P450(s) as suggested by our study. It is possible that the female requires estradiol for feminization. The hypophysectomized male rat appears to require only relatively low doses of either GH (5 ug/hr used in our study) or estradiol for feminization; whereas the hypophysectomized female may need larger doses of GH (10 ug/hr used in our study) or both GH and estradiol to achieve feminization. In the intact female it has been reported that estradiol may exert direct effects on the liver (ie., decrease hepatic enzymes) (Lax et al., 1983) in addition to its effects on the hypothalamic-pituitary axis which results in the stimulation of GH secretion (Colby, 1980; Gross, 1980). Therefore, since circulating levels of estradiol are very low in the hypophysectomized female, full feminization may not be able to be attained.

In conclusion, the results of this study show that GH feminizes (ie., decreases) EMDM activity in the hypophysectomized male rat by decreasing the synthesis of SP-apocytochrome P450, probably at the level of

translation. In the hypophysectomized female, GH feminizes EMDM activity by inhibiting the synthesis of FP-apocytochrome P450.

LITERATURE CITED

- Axelrod, J. 1956. The enzymatic N-demethylation of narcotic drugs. *J. Pharm. Exper. Ther.* 117: 322.
- Baxter, R.C. and Zaltsman, Z. 1984. Induction of hepatic receptors for GH (growth hormone) and prolactin by GH infusion is sex independent. *Endocrinology*. 115: 2009.
- Bend, J.R. and Hook, G.E.R. 1975. Hepatic and extrahepatic mixed function oxidases. In: Handbook of Physiology, section 9: Reactions to environmental agents. D. Lee, H.L. Falk, S.D. Murphy, S.R. Geiger (eds). Amer. Physiol. Society, Bethesda, Maryland. Ch. 26 pp 419-436.
- Bhat, K.S., Sardana, M.K., and Padmanaban, G. 1977. Role of haem in the synthesis and assembly of cytochrome P-450. *Biochem. J.* 164: 295.
- Chao, H. and Chung, L.W.K. 1982. Neonatal imprinting and hepatic cytochrome P-450-I. Immunochemical evidence for the presence of a sex-dependent and neonatally imprinted form(s) of hepatic cytochrome P-450. *Molecul. Pharm.* 21: 744.
- Cheng, K.C. and Schenkman, J.B. 1982. Purification and characterization of two constitutive forms of rat liver microsomal cytochrome P-450. *J. Biol. Chem.* 257: 2378.
- Chung, L.W.K. 1977. Characteristics of neonatal androgen-induced imprinting of rat hepatic microsomal monooxygenases. *Biochem. Pharm.* 26: 1979.
- Chung, L.W.K. and Chao, H. 1980. Neonatal imprinting and hepatic cytochrome P-450. 1. Comparison of testosterone hydroxylation in a reconstituted system between neonatally imprinted and nonimprinted rats. *Molecular Pharmacology*. 18: 543.
- Cochin, J. and Axelrod, J. 1959. Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphins and normorphine. *J. Pharmacol. Exp. Ther.* 125: 105.
- Colby, H.D. 1980. Regulation of hepatic drug and steroid metabolism by androgens and estrogens. In: J.A. Thomas and R.L. Singhal (eds): Advances

- in Sex Hormone Research. Baltimore. Munich: Urban and Schwarzenberg, pg 27.
- Correia, M.A. and Meyer, U.A. 1975. Apocytochrome P-450: Reconstitution of functional cytochrome with hemin in vitro. Proc. Nat. Acad. Sci. USA 72(1): 400.
- Dannan, G.A., Guengerich, F.P., Kaminsky, L.S., and Aust, S.D. 1983. Regulation of cytochrome P-450. Immunochemical quantitation of eight isozymes in liver microsomes of rats treated with polybrominated biphenyl congeners. J. Biol. Chem. 258: 1282.
- Davies, D.S., Gigon, P.L., and Gillette, J.R. 1968. Sex differences in the kinetic constants for the N-demethylation of ethylmorphine by rat liver microsomes. Biochem. Pharmacol. 17: 1865.
- Eden, S. 1979. Age- and sex-related differences in episodic growth hormone binding component of isolated rat hepatocytes. Biochem. 19: 3293.
- Einarsson, K., Gustafsson, J., and Stenberg, A. 1973. Neonatal imprinting of liver microsomal hydroxylation and reduction of steroids. J. Biol. Chem. 248: 4987.
- Eisenfeld, A.J., Aten, R., Weinberger, M., Haselbacher, G., Halpern, K., and Krakoff, L. 1976. Estrogen receptor in the mammalian liver. Sci. 191: 862.
- El Defrawy El Masry, S., Cohen, G.M., and Mannering, G.J. 1974. Sex-dependent differences in drug metabolism in the rat. I. Temporal changes in the microsomal drug-metabolizing systems of the liver during sexual maturation. Drug Metab. Dispos. 2(3): 267.
- Garner, R.C. and McLean, A.E.M. 1969. Separation of haem incorporation from protein synthesis in liver microsomes. Biochem. Biophys. Res. Commun. 37(6): 883.
- Gillette, J.R., Davis, D.C., and Sasame, H.A. 1972. Cytochrome P-450 and its role in drug metabolism. Am. Rev. Pharmacol. 12: 57.
- Gomori, G. 1955. Preparation of buffers for use in enzyme studies. Methods in Enzymology. 1: 137.

- Goth, A. 1984. Medical Pharmacology. C.V. Mosby Co., St. Louis, Miss. pg 29-40
- Gross, D.S. 1980. Role of somatostatin in the modulation of hypophysial growth hormone production by gonadal steroids. *Am. J. Anat.* 158: 507.
- Guengerich, F.P., Dannan, G.A., Wright, S.T., and Martin, M.V. 1982. Purification and characterization of microsomal cytochrome P-450s. *Xenobiotica* 12: 701.
- Gustafsson, J.A., Housette, A., Stenberg, A., and Wrangé, O. 1975. High-affinity binding of 4-androstene-3,17-dione in rat liver. *Biochem.* 14: 3942.
- Gustafsson, J.A. and Stenberg, A. 1976. On the obligatory role of the hypophysis in sexual differentiation of hepatic metabolism in rats. *Proc. Natl. Acad. Sci.* 73: 1462.
- Gustafsson, J.A. and Skett, P. 1978. Precocious 'feminization' of rat liver enzymes in the presence of an ectopic pituitary. *J. Endo.* 76: 187.
- Gustafsson, J.A., Enroth, P., Haglund, B., Hokfelt, T., Mode, A., Skett, P., and Wrangé, O. 1979. Sexual differentiating actions of steroids on the hypothalamic-pituitary-liver axis. In: Central Regulation of the Endocrine System. K. Fuxe, T. Hokfelt, and R. Luft (eds). Plenum Press N.Y. pg. 315.
- Gustafsson, J.A., Mode, A., Norstadt, G., Hokfelt, T., Sonnenschein, C., Eneroth, P., and Skett, P. 1980. The hypothalamo-pituitary-liver axis: A new hormonal system in control of hepatic steroid and drug metabolism. In: Biochemical Actions of Hormones, Vol VII. G. Litwack ed. Academic Press. N.Y. N.Y. pg. 48-83.
- Gustafsson, J.A., Mode, A., Norstadt, G., and Skett, P. 1983a. Sex steroid induced changes in hepatic enzymes. *Ann. Rev. Physiol.* 45: 51.
- Gustafsson, J.A., Eden, S., Eneroth, P., Hokfelt, T., Isaksson, O., Jansson, J., Mode, A., and Norstedt, G. 1983b. Regulation of sexually dimorphic hepatic steroid metabolism by the somatostatin-

- growth hormone axis. *J. Steroid Biochem.* 19: 691.
- Gustafsson, J.A., Mode, A., Norstedt, G., Eneroth, P., and Hokfelt, T. 1983c. Growth hormone - A regulator of the sexually differentiated steroid metabolism in rat liver. *Prog. in Clin. and Biol. Res.* 135: 37. In: Developmental Biology. S.M. MacLeod, A.B. Okay, and S.P. Spielberg eds. Alan R. Liss Inc., N.Y.
- Hadley, M.E. 1984. Endocrinology. Prentice Hall Inc., New Jersey pg 266.
- Hizuka, N., Gorden, P., Lesniak, M.A., Obberghen, E.V., Carpenter, J.L., and Orci, L. 1981. Polypeptide hormone degradation and receptor regulation are coupled to ligand internalization. *J. of Biol. Chem.* 236(9): 4591.
- Hughes, J.P. and Friesen, H.G. 1985. The nature and regulation of the receptors for pituitary GH. *Ann. Rev. Physiol.* 47: 469.
- Imai, Y., Ito, A. and Sato, R. 1966. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. *J. Biol. Chem.* 60: 417.
- Jansson, J., Ekberg, S., Isaksson, O.G.P., and Eden, S. 1984. Influence of gonadal steroids on age- and sex-related secretory patterns of growth hormone in the rat. *Endocrinology*. 114: 1287.
- Jansson, J., Ekberg, S., Isaksson, O., Mode, A., and Gustafsson, J. 1985a. Imprinting of growth hormone secretion, body growth, and hepatic steroid metabolism by neonatal testosterone. *Endocrinology*. 117: 1881.
- Jansson, J., Eden, S., and Isaksson, O. 1985b. Sexual dimorphism in the control of growth hormone secretion. *Endocrine Reviews*. 6: 128.
- Kamataki, T., Maida, K., Yamazoe, Y., Nagai, T., and Kato, R. 1983. Sex difference of cytochrome P-450 in the rat: Purification, characterization, and quantitation of constitutive forms of cytochrome P-450 from liver microsomes of male and female rats. *Arch. Biochem. Biophys.* 225: 758.
- Kamataki, T., Shimada, M., Maeda, K., and Kato, R. 1985. Pituitary regulation of sex-specific forms

- of cytochrome P-450 in liver microsomes of rats. Biochem. Biophys. Res. Comm. 130: 1247.
- Kato, R. and Gillette, J.R. 1965. Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. J. Pharm. Exp. Ther. 150: 279.
- Kato, R. and Onoda, K. 1970. Studies on the regulation of the activity of drug oxidation in rat liver microsomes by androgen and estrogen. Biochem. Pharm. 19: 1649.
- Kato, R. 1974. Sex-related differences in drug metabolism. Drug Metab. Rev. 3(1): 1.
- Kato, R. 1979. Characteristics and differences in the hepatic mixed function oxidases of different species... Pharm. Ther. 6(1): 41.
- Keppel, G. 1982. Design and Analysis: A Researcher's Handbook. 2nd edition. Prentice-Hall Inc., Englewood Cliffs, New Jersey.
- Korner, A. 1968. Anabolic action of growth hormone. Annals NY Acad. Sci. 148: 408.
- Korner, A. 1969. Growth hormone effects on RNA and protein synthesis in liver. J. Cell. and Comp. Physiol. 66: 153.
- Kostyo, J.L., and Nutting, D.F. 1974. Growth hormone and protein metabolism. In: E. Knobil, W. Sawyer (eds) Handbook of Physiology, sect 7, part 2, American Physiological Society, Washington D.C., vol 4:187.
- Kramer, R.E., Greiner, J.W., and Colby, H.D. 1975. Divergence of growth hormone actions on hepatic drug metabolism in the presence and absence of the pituitary gland. Life Sciences. 17: 779.
- Kramer, R.E. and Colby, H.D. 1976. Feminization of hepatic steroid and drug metabolizing enzymes by growth hormone in male rats. J. Endocrinology. 71: 449.
- Kramer, R.E., Greiner, R.C., Rumbaugh, R.C., Sweeney, T.D., and Colby, H.D. 1978a. Relation of the gonadal hormones to growth hormone actions on hepatic drug metabolism in rats. J. Pharm. Exp. Ther. 204: 247.

- Kramer, R.E., Greiner, R.C., Rumbaugh, R.C., Sweeney, T.D., and Colby, H.D. 1978b. Requirement of the pituitary gland for gonadal hormone effects on hepatic drug metabolism in rats. *J. Pharm. Exp. Therap.* 208: 19.
- Levin, W. and Kuntzman, R. 1969. Biphasic decrease of radioactive hemoprotein from liver microsomal CO-binding particles. *J. Biol. Chem.* 367: 1.
- Levin, W., Alvares, A.P., and Kuntzman, R. 1970. Distribution of radioactive hemoprotein and CO-binding pigment in rough and smooth endoplasmic reticulum of rat liver. *Arch. Biochem. Biophys.* 130: 230.
- Levin, W. and Ryan, D. 1975. Age and sex differences in the turnover of rat liver cytochrome P-450: The role of neonatal imprinting. *Basic and Therapeutic Aspects of Perinatal Pharmacology.* 265.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265.
- Lu, A.Y. and Coon, M.J. 1968. Role of hemoprotein P-450 in fatty acid ω -hydroxylation in a soluble enzyme system from liver microsomes. *J. Biol. Chem.* 243: 1331.
- Lui, E.M.K., Slaughter, S.R., Philpot, R.M., and Lucier, G.W. 1982. Endocrine regulation of cadmium-sensitive cytochrome P-450 in rat liver. *Molecul. Pharm.* 22: 795.
- MacGeoch, C., Morgan, E.T., Halpert, J., and Gustafsson, J. 1984. Purification, characterization, and pituitary regulation of the sex-specific cytochrome P-450 15B-hydroxylase from liver microsomes of untreated female rats. *J. Biol. Chem.* 259: 15433.
- MacGeoch, C., Morgan, E.T., and Gustafsson, J. 1985. Hypothalamo-pituitary regulation of cytochrome P-450 apoprotein levels in rat liver. *Endocrinology.* 117: 2085.
- MacKinnon, M., Sutherland, E., and Simon, F.R. 1977. Effects of ethinyl estradiol on hepatic microsomal proteins and the turnover of cytochrome P-450. *J.*

Lab. Clin. Med. 90(6): 1096.

- Maeda, K., Kamataki, T., Nagai, T., and Kato, R. 1984. Postnatal development of constitutive forms of cytochrome P-450 in liver microsomes of male and female rats. *Biochemical Pharmacology*. 33: 509.
- Matsuura, S., Fujii-Kuriyama, Y., and Tashiro, Y. 1978. Immunoelectron microscope localization of cytochrome P-450 on microsomes and other membrane structures of rat hepatocytes. *J. Cell. Biology* 78: 503.
- Mayer, S.E., Melmon, K.L., and Gillman, A.G. 1980. The dynamics of drug absorption, distribution, and elimination. In: The Pharmacological Basis of Therapeutics. Gilman, A.G., Goodman, L.S., and Gilman, A. (eds). MacMillan Publishing Co. Inc., New York. Ch.1, p. 21.
- Mode, A., and Norstedt, G. 1982. Effects of gonadal steroid hormones on the hypothalamo-pituitary-liver axis in the control of sex differences in hepatic steroid metabolism in the rat. *J. Endocrinology* 95: 181.
- Mode, A., Norstedt, G., Simic, B., Eneroth, P., and Gustafsson, J. 1981. Continuous infusion of growth hormone feminizes hepatic steroid metabolism in the rat. *Endocrinology*. 108:2103.
- Mode, A., Gustafsson, J., Jansson, J., Eden, S., and Isaksson, O. 1982. Association between plasma level of growth hormone and sex differentiation of hepatic steroid metabolism in the rat. *Endocrinology*. 111: 1692.
- Mode, A., Norstedt, G., Eneroth, P., and Gustafsson, J. 1983. Purification of liver feminizing factor from rat pituitaries and demonstration of its identity with growth hormone. *Endocrinology*. 113: 1250.
- Morgan, E.T., MacGeoch, C., and Gustafsson, J. 1985a. Sexual differentiation of cytochrome P-450 in rat liver. Evidence for a constitutive isozyme as the male-specific 16 α -hydroxylase. *Mol. Pharm.* 27: 471.
- Morgan, E.T., MacGeoch, C., and Gustafsson, J. 1985b. Hormonal and developmental regulation of expression of the hepatic microsomal steroid 16 α -

- hydroxylase cytochrome P-450 apoprotein in the rat.
J. Biol. Chem. 260: 11895.
- Murad, A., and Haynes, 1980. Adenohypophyseal hormones and related substances. In: The Pharmacological Basis of Therapeutics. Gilman, A.G., Goodman, L.S., and Gilman, A. (eds). MacMillan Publishing Co. Inc., New York p. 1369.
- Nebert, D. 1981. Genetic mechanisms controlling the induction of polysubstrate monooxygenase (P-450) activities. Ann. Rev. Pharm. and Toxicol. 21: 431.
- Nisimoto, Y., Kinoshita, K.Jr., Ikegami, A., Kawai, N., Ichihara, I., and Shibata, Y. 1983. Possible association of NADPH-cytochrome P-450 reductase and cytochrome P-450 in reconstituted phospholipid vesicles. Biochem. 22: 3586.
- Norstedt, G. 1982. A comparison between the effects of GH on prolactin receptors and estrogen receptors in rat liver. Endocrinology. 110: 2107.
- Norstedt, G., Anderson, G., and Gustafsson, J. 1984. Growth hormone induction of lactogenic receptors at intracellular sites in male rat liver. Endocrinology. 115: 672.
- Omura, T. and Sato, R. 1964. The carbon monoxide binding pigment of liver microsomes II. Solubilization, purification, and properties. J. Biol. Chem. 239: 2379.
- Phillips, A.H. and Langdon, R.G. 1962. Hepatic triphosphopyridine nucleotide-cytochrome c reductase: isolation, characterization and kinetic studies. J. Biol. Chem. 237: 2652.
- Posner, B.I., Bergeron, J.J.M., Josefsberg, Z., Khan, M.N., Khan, R.J., Patel, B.A., Sikstrom, R.A. and Verma, A.K. 1981. Res. Prog. Horm. Res. ed. R.O. Greep, vol 37: 559.
- Postel-Vinay, M.C., Kayser, C. and Desbuquois, B. 1982. Fate of injected human growth hormone in the female rat liver microsomes. Endocrinology. 111: 244.
- Ranke, M.B., Stanely, C.A., Tenore, A., Rodbard, D., Bongiovanni, A.M., and Parks, J.S. 1976. Characterization of somatogenic and lactogenic binding sites in isolated rat hepatocytes.

Endocrinology. 99(4):1033.

- Robson, P.M., Goll, D.E. and Temple, M.J. (1968). Determination of proteins in Tris buffer by the biuret reaction. *Anal. Biochem.* 24: 339.
- Rumbaugh, R.C., and Colby, H.D., 1980. Is growth hormone the pituitary feminizing factor mediating the actions of estradiol on hepatic drug and steroid metabolism? *Endocrinology.* 107: 719.
- Ryan, D.E., Thomas, L.M., Reik, L.M., and Levin, W. 1982. Purification, characterization and regulation of five rat hepatic microsomal cytochrome P-450 isozymes. *Xenobiotica.* 12: 727.
- Ryan, D.E., Iida, S., Wood, A.W., Thomas, P.E., Lieber, C.S. and Levin, W. 1984. Characterization of three highly purified cytochromes P-450 from hepatic microsomes of adult male rats. *J. Biol. Chem.* 259: 1239.
- Schenkman, J.B., Frey, I., Remmer, H., and Estabrook, R.W. 1967. Sex differences in drug metabolism by rat liver microsomes. *Molec. Pharm.* 3: 516.
- Skett, P. and Gustafsson, J.A. 1980. Imprinting of enzyme systems of xenobiotic and steroid metabolism. Reviews in Biochemical Toxicology. In: Hodgson, E., Bend, J., and Philpot, R. (eds). Elsevier/North Holland, New York, p. 27.
- Shiraki, H. and Guengerich, F.P. 1984. Turnover of membrane proteins: Kinetics of induction and degradation of seven forms of rat liver microsomal cytochrome P-450, NADPH cytochrome P-450 reductase, and epoxide hydrolase. *Arch. Biochem. Biophys.* 235(1): 86.
- Sokal, R.R. and Rohlf, F.J. 1981. *Biometry. The principles and practice of statistics in biological research.* 2nd edition. W.H. Freeman and Co. New York.
- Spencer, E.M. 1979. Synthesis by cultured hepatocytes of somatomedin and its binding protein. *FEBS letters.* 99 157.
- Strobel, H.W., Lu, A.Y., Heidema, J., and Coon, M.J. 1970. Phosphatidylcholine requirement in the enzymatic reduction of hemoprotein P-450 and in fatty acid, hydrocarbon and drug hydroxylation.

- J. Biol. Chem. 245: 4851.
- Van Wyk, J.J. and Underwood, L.E. 1978. The somatomedins and their actions. In. Biochemical Actions of Hormones. G. Litwack ed. vol 5: 101.
- Virgo, B.B. 1983. Endogenous growth hormone and the regulation of hepatic drug oxidation in the male rat. Developmental Pharmacology. Alan R. Liss Inc. N.Y. pg 379.
- Virgo, B.B. 1985. Effects of somatostatin and testosterone on the hepatic monooxygenase system in castrated male rats. Drug Metabolism and Disposition. 13: 1.
- Waxman, D.J. 1984. Rat hepatic cytochrome P-450 isoenzyme 2c: Identification as a male-specific developmentally induced steroid 16 α -hydroxylase and comparison to a female-specific cytochrome P-450 isoenzyme. J. Biol. Chem. 259(24): 15481.
- Waxman, D.J., Dannan, G.A., and Guengerich, F.P. 1985. Regulation of rat hepatic cytochrome P-450: Age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex specific isoenzymes. Biochemistry. 24: 4409.
- Welch, R.M. 1979. Toxicological implications of drug metabolism. Pharmacol. Rev. 30(4): 457.
- Wilson, J.T. 1973. Growth hormone modulation of liver drug metabolic enzyme activity in the rat-1. Effect of the hormone on the content and rate of reduction of microsomal cytochrome P-450. Biochem. Pharm. 22: 1717.
- Wilson, J.T. and Spelsberg, T.C. 1976. Growth hormone and drug metabolism. Acute effects on microsomal mixed-function oxidase activities in rat liver. Biochem. J. 154: 433.

VITA AUCTORIS

Name: Birgit M. Vockentanz

Born: February 17, 1962, Schwabach, West-Germany

Elementary Education: Walker Road Public School
Windsor, Ontario
(1967-1975)

Secondary Education: Walkerville S.S.
Windsor, Ontario
(1975-1980)

University Education: University of Windsor
Windsor, Ontario
M.Sc. commenced in 1984
Hon. B.Sc. in 1984